Axial loading as a countermeasure to microgravity-induced deconditioning; effects on the spine and its associated structures

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Abstract

Prolonged spinal unloading in microgravity has been associated with stature elongation and increased risk of intervertebral disc (IVD) herniation, particularly in the lumbar spine. Novel countermeasures to reintroduce axial loading in space are therefore required. This PhD aimed to evaluate the impact of a novel axial loading countermeasure upon stature, spinal structure and functionality, both when static and during motion, utilising a microgravity analogue. Five studies were conducted using novel 'hyper-buoyancy flotation' (HBF) as the microgravity analogue, enabling accessible 'unloading' for the evaluation of the European Space Agency's 'SkinSuit' (Mk VI) which imparts low-level axial loading (~20% bodyweight).

Chapter 3 evaluated HBF's ability to induce stature elongation. Two groups underwent 4h (n=14) or 8h (n=14) HBF, resulting in a stature elongation (2.1cm), which was greater than that reported in comparable analogues. Chapter 4 (n=9) demonstrated that Mk VI SkinSuit wear attenuated stature (1.7±0.5cm vs. 2.1±0.4cm) and partial lumbar (L1-L3) IVD expansion following 8h HBF, whilst Chapter 5 (n=6) found that SkinSuit loading reduced stature elongation and lumbar length (L1-S1:17.8±1.0 vs. 18.1±0.8cm), presumably through a combination of minor IVD compression and an increase in lumbar lordosis, after 8h HBF. Chapters 6 and 7 evaluated the effects of 4h SkinSuit reloading following 8h unloading. In Chapter 6 (n=8), immediate effects of SkinSuit reloading were observed on stature and in several lumbar IVDs measured using ultrasound (0.2-1.0mm reduction: L2-S1 IVDs). In Chapter 7 (n=8), it was found using quantitative fluoroscopy, that SkinSuit reloading resulted in minor reductions in intervertebral restraint during passive flexion and in reductions in lumbar IVD height (L3/4:-0.44mm and L4/5:-0.34mm) measured using MRI.

These pilot studies suggest that HBF holds promise as a microgravity analogue. The SkinSuit imparted low-level axial loading that consistently attenuated stature. Minor IVD compression was observed which may have led to small attenuations in intervertebral restraint during flexion. Further testing in space and with analogues is recommended, to determine the effectiveness of cumulative wear at mitigating spinal deconditioning.

Table of contents

List of figures

List of tables

Glossary of abbreviations

Microgravity	μG
Gravity	G
Gravity in a down or upwards (shoulder <->foot) direction	Gz (+/-)
Head down tilt	HDT
Hyper-buoyancy floatation	HBF
Magnetic resonance imaging	MRI
Quantitative fluoroscopy	QF
Dual x-ray absorptiometry	DEXA
Intra-abdominal pressure	IAP
Electromyography	EMG
Cervical spine (1-7)	C#
Thoracic spine (1-12)	T#
Lumber spine (1-5)	L#
Intervertebral disc	IVD
Herniated nucleus pulposus	HNP
Extravehicular	EVA
International space station	ISS
European space agency	ESA
National aeronautics and space administration	NASA
Advanced resistance exercise device	ARED
Combined operational load bearing external resistance treadmill	COLBERT
Cycle ergometer vibration isolated device	CEVIS

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Chapter 1.Introduction

The evolution of human life on Earth has been driven by an imperative to resist the force of gravity. This has shaped every facet of our race in our development from a nomadic species to a global, spacefaring race. The opposition of gravity also strongly affects the behaviour of the human body at both the cellular and systemic levels. At the cellular level, alterations in mechanical stress, that can be facilitated by gravity load/unloading, affects cellular morphology, development and genetic expression (Monici *et al.*, 2011). Imbalances in the load/unloading of tissues precipitates structural remodelling and expression of apoptosis pathways (Jin *et al.*, 2013). At the systemic level, the human body processes afferent and efferent signals to maintain balance in its systems. An example is the continued delivery of blood to brain by the cardiovascular system. Prolonged exposure to unloading can lead to system failures upon the reintroduction of loading i.e. with return to Earth from space, where the body attempts to compensate to the change in mechanical stress (Buckey Jr. *et al.*, 1996). Through studying the impact of altered gravity upon the body, further understanding into the aetiology of terrestrial states of imbalance are acquired, with the aim to develop protective countermeasures.

In the study of gravity's effect upon the body, appreciation of both the planetary gravitational constant and the constituent components of the applied acceleration force are required. On Earth, the gravitational acceleration is termed g, and has a value of $9.81ms^{-2}$. The unit G is the ratio of an applied acceleration to the gravitational constant, so G=acceleration/g (Glaister and Prior, 1999). In the expression of G, the axis (Gx, Gy, Gz) direction (positive [Gz+] towards the feet and negative [Gz-] towards the head) and magnitude (1G, 2G, etc) of the applied acceleration force is described. As an example, standing upright on Earth the body experiences 1Gz acting downwards towards the feet loading the musculoskeletal system. When supine, the axis is changed to Gx, acting anteriorly to posterior facilitating an unloading of the weight-bearing skeleton. Gy forces act laterally along the sagittal plane (shoulder to shoulder) and would be experienced when side lying or during aerobatic manoeuvres. Variations in the magnitude, axis and direction thus act to create a cycle of loading and unloading upon the body.

It is within this cycle of axial loading and unloading that the spine and its associated structures have developed to facilitate movement and locomotion (Le Huec *et al.*, 2011). However, with severe disruptions in this cycle incurred from the transition from Earth's gravitational force to the microgravity environment of space, deleterious effects can occur, the focus of which for this thesis is the spine. Significant stature elongation, back pain and increased risk of intervertebral disc herniation have been documented in astronauts, attributed to the loss of the mechanical loading stimulus that is imparted by G forces when on Earth. Therefore, novel methods are required to reintroduce axial loading in space that meet the operational requirements of low volume, mass and power consumption. Evaluation of proposed countermeasures requires the use of suitable analogue platforms on Earth that can provide both accessibility and utility. Thus, the primary aim of this thesis was to evaluate the effect of an axial loading countermeasure, the Mk VI SkinSuit upon the spine with the use of a novel microgravity analogue.

In the literature review (Chapter 2), previous research investigating the effects of loading and unloading upon stature and the spine are discussed, with attention on the documented effects of human spaceflight upon the spine and its associated structures. Current pertinent countermeasures and utilised analogues are presented alongside the European Space Agency's Mk VI SkinSuit, which is the focus of evaluation within this thesis. The Mk VI SkinSuit imparts low-level axial loading, shoulder to foot through a bi-directional elastic weave, which is described further in Chapter 4.

Chapter 3 presents the studies undertaken to determine the suitability of a novel analogue platform Hyper-Buoyancy Flotation (HBF), as an analogue to induce significant stature elongation. It is comprised of two study lengths upon the HBF, the first 4h and the second 8h, where the amount of elongation experienced and subjective comfort were assessed. This was compared with literature from other spaceflight analogues and from 8h sleep studies.

Chapters 4 through to 7 explore how the Mk VI SkinSuit affects the documented responses to unloading induced by HBF. These studies used the participants as their own controls, where measurements from a control (unloaded condition) and a partial axial (Gz) loaded condition via the Mk VI SkinSuit are compared. In Chapter 4, the first pilot study with the Mk VI SkinSuit, an 8h HBF session was performed twice, once when wearing gym clothes and the other when wearing the Mk VI SkinSuit. Stature and subjective measurements were taken as per Chapter 3, followed by a sagittal dual x-ray absorptiometry (DEXA) scan of the lumbar spine to assess potential compression of the intervertebral discs. Owing to metallic components in the SkinSuit, DEXA was chosen as the imaging modality in this initial pilot.

Chapter 5 builds upon Chapter 4 by investigating the effects of 8h loaded Mk VI SkinSuit wear on the whole spine, with a focus on the lumbar spine. For this Chapter, the Mk VI SkinSuit was modified to facilitate magnetic resonance imaging (MRI) taking sagittal slices of the spine to measure length, intervertebral disc heights (cervical to lumbar) and lumbar lordosis, in a comparable manner to other load/unloading studies (Kimura *et al.*, 2000; Belavý *et al.*, 2011).

Chapters 6 and 7 evaluate the effects of reloading the spine, after 8h overnight unloading. Chapter 6 pilots a NASA ultrasound protocol used on the international space station (ISS) (Marshburn et al. 2014) to take repeated measures of cervical and lumbar anterior intervertebral disc height with 8h HBF unloading followed by 4h SkinSuit reloading. Chapter 7 utilises the piloted protocol from Chapter 6 to investigate the effects of 4h SkinSuit reloading on lumbar geometry and kinematics with MRI and quantitative fluoroscopy. At the end of each of the experimental Chapters (3-7) an image chronicling the parallel testing and incorporation of the SkinSuit with the ESA astronauts for spaceflight assessment is included, for operational context.

Finally, Chapter 8 discusses the overall contributions of the thesis and novel implications alongside recommendations for future research.

Chapter 2. Review of literature

A short description of the spine and the supporting structures is first presented for background information (Section 2.01). In the subsequent literature review, context given to the processes associated with the ‘normal’ loading and unloading cycles of the spine experienced on Earth. The impact of disrupting these cycles through excessive loading and prolonged unloading is then explored, through which an introduction to the known effects of the space environment is provided. The issues experienced by astronauts and the work done to attempt to counter the negative effects associated with microgravity are discussed followed by analogue platforms, that assist with the study of the impact of the space environment on humans, on Earth. Finally, the European Space Agency’s SkinSuit project, designed to provide partial axial reloading to astronauts in space is presented followed by the specific aims of this PhD.

Section 2.01 The spine and its principal structural components

The vertebrae and the intervertebral discs (IVDs)

The spine is typically comprised of 33 vertebrae (though variation does exist), which become larger in response to the weight placed upon them, as the spine projects from the cranium to the pelvis supporting an upright position against gravity. Each vertebra is formed of a core, spongy, cancellous bone, whilst the surrounding outer body is composed of dense cortical bone. The superior and inferior surfaces attach to the IVD thus are smooth and are made of cancellous bone to enable nutrient transfer (Figure 1). Joined to the vertebral bodies are the processes, vertebral arches and apophyseal joints, performing several functions depending on regions, including providing protective channels for the spinal cord, attachment points for the muscular system, leveraging of movement, resistance and protection from sheer forces, axial rotation and excessive flexion (Adams and Hutton, 1983) and provision of an interlocking system with their neighbouring vertebrae (Drake, Vogl and Mitchell, 2010) (Figure 1). In-between each non-fused vertebra is an intervertebral disc (IVD), comprised of two main components: the nucleus pulposus and the annulus fibrosis, which are sandwiched between the superior and inferior vertebral endplates that are composed of a connecting cartilaginous endplate that is loosely bonded to the cortical bone section of the vertebrae enabling diffusion (Raj, 2008; Cao *et al.*, 2017).

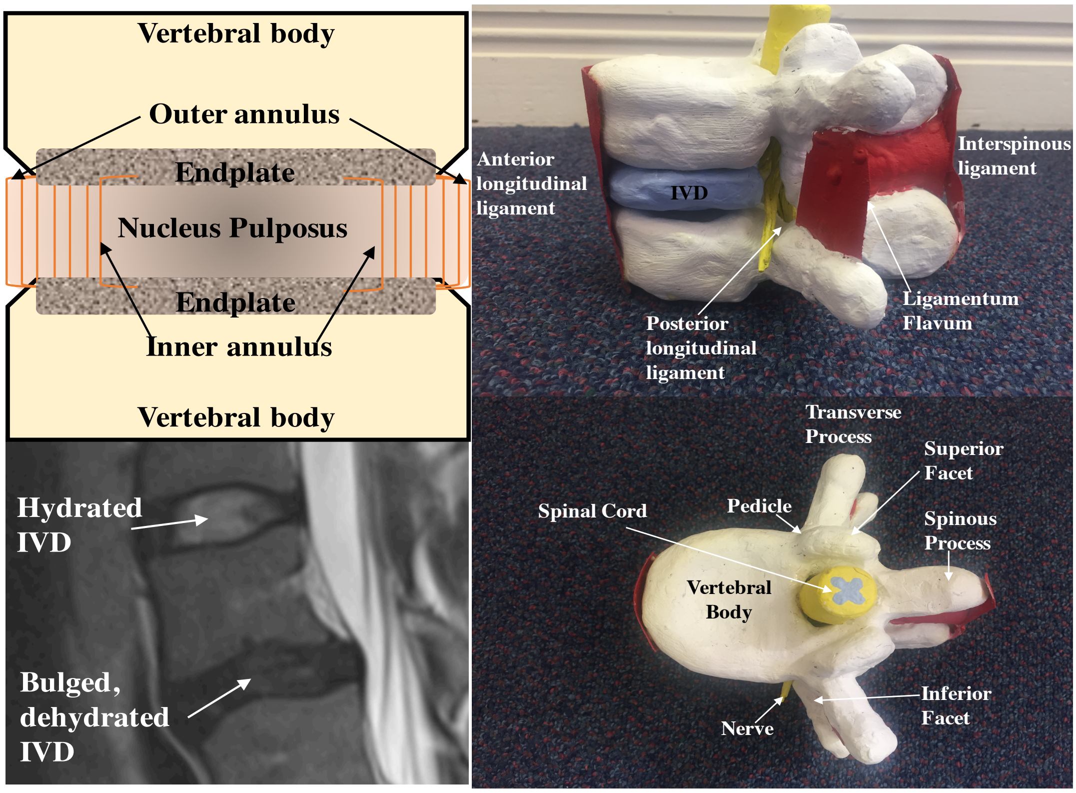


Figure 1. Top left: Schematic of the intervertebral disc and its components. Bottom left: an example of a lumbar spine using T2-weighted, sagittal plane magnetic resonance imaging, showing a 'healthy, hydrated (light colour) disc' and a dehydrated (dark colour) bulging' disc. Image credit (left) King's College London. Right – MRI: A labelled construction of a lumbar segment (right). Credit for physical construction of the lumbar segment goes to the first-year chiropractic students at AECC.

The IVD is made up of a gel like centre called the nucleus pulpous, composed primarily of the hydrophilic protein proteoglycan, type II collagen proteins and water, which binds to the proteoglycan molecules (Ghannam *et al.*, 2017) (Figure 1). Type II collagen fibres forms a mesh providing structure for the nucleus, whilst non-collagenous proteins and elastin and make up most of remaining constituent parts of the nucleus (Newell *et al.*, 2017). Type II collagen being associated with cartilage structures which imparts considerable strength and compressibility to resist large deformations (Lodish *et al.*, 2000).

Surrounding the nucleus pulpous is the inner and outer annulus fibrosis made up of bundles of fibres arranged in lamellae layers positioned both radially and circumferentially, with bundles orientated at multiple angles to provide structural support against compressive forces, torsional stresses and shear around each vertebral/IVD section (Smith and Fazzalari, 2009; Adams *et al.*, 2013). These lamellae are composed primarily of type 1 collagen fibres, type 1 being associated with tendon like structures providing great tensile strength and stretch without breaking (Lodish *et al.*, 2000). Accompanying the high percentage of collagen fibres are a lower percentage of proteoglycans and other cell types i.e. elastin (Ghannam *et al.*, 2017). The fibres from the inner annulus extrude into the cartilaginous/cancellous sections of the endplate, whilst the outer attach to the outer sections of the vertebrae formed of cortical bone but also the anterior and posterior ligaments (Newell *et al.*, 2017) (Figure 1). Along the spinal column are ligaments designed to support the distribution of forces, provide structure, regulate degrees of movements at certain vertebral levels (i.e. lower thoracic) and support alignment. The endplates meanwhile contain the micro vessel network. It is through this network, coupled with the surrounding vasculature, that nutrient transfer is facilitated via diffusion (Figure 1). The bi-directional diffusion of nutrients initiates in the small capillaries around the subchondral bone, through the matrix of the endplate before going into the nucleus pulposus. This diffusional process has been traced using a gadolinium-based MRI contrast agent, which observed that up to 6 hours was required before this process was complete (Rajasekaran *et al.*, 2004).

These structures react to loading stimulus affecting their remodelling and adaptation. If the stimulus is too low, as the case is in microgravity, the bone will weaken due to the loss of remodelling mechanoreceptor stimulus (Richter *et al.*, 2017). In general this is in accordance with Wolff's mathematical law, though as regional variations, environmental factors (genetics, age) and repair processes affect recorded bone remodelling, this is also referred to as 'Bone functional adaptation' (Ruff, Holt and Trinkaus, 2006). A recommendation for the weight bearing bones states a minimum remodelling threshold of between 1,000–1,500 microstrains; ~2 kg/mm^2 from countermeasures is required to optimise the absorption/remodelling paradigm (Richter *et al.*, 2017). How these stresses act upon the spine is important to understand as is not simply a consequence of compressive loading imparted by gravity and ground reaction forces, but also from one of the largest actors on the spine, the interaction from the musculoskeletal system.

__The spine's muscular system__

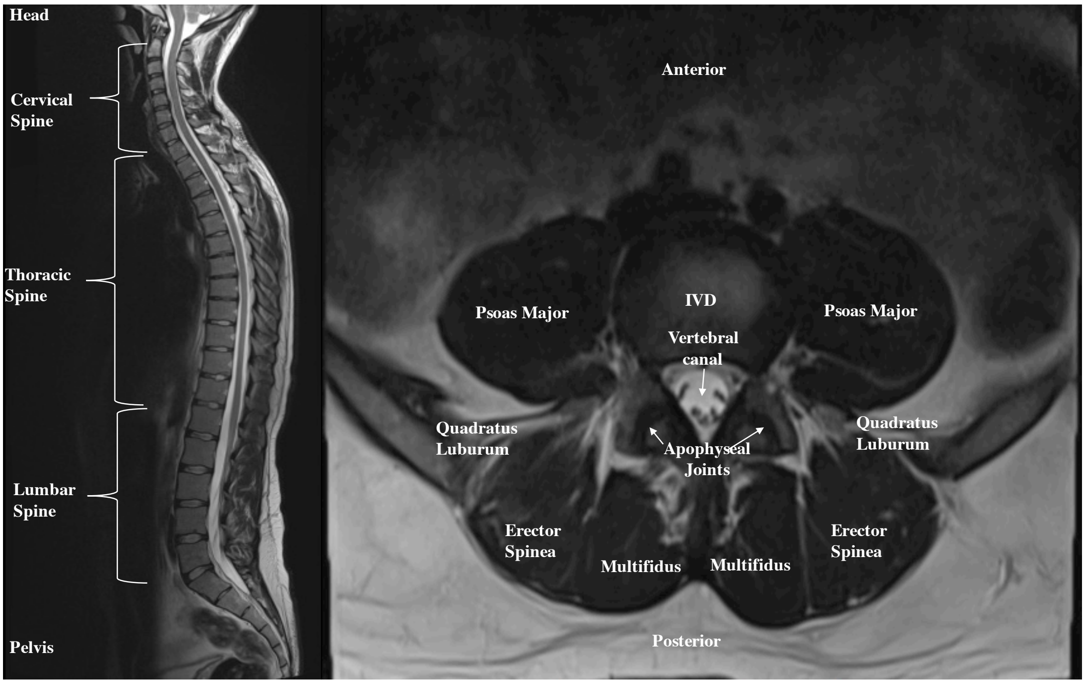


Figure 2. Left - MRI of the entire spine using a T2 sagittal slice to include spinal regions. Right - Axial slice at L4/L5 to show lumbar muscles and apophyseal joints

Supporting the spinal column is a complex web of muscles endowing the spine with the structural support required for triplane movement including, flexion and extension, lateral bending and rotation (Figure 2). These muscles through their interaction (insertion and origin) with the vertebral processes facilitate the translation of stresses along the vertebral bodies and the application of forces along and through the IVD. These are critical to overall functionality and stability, which if compromised can lead to potential injury of the spinal unit, particularly in the lumbar region, on which the thesis is predominantly focussed. It is important to note that anatomical variations are common between individuals, with some studies reporting (generally) minor conflictions in origins and insertions of muscle bundles. Recently, whilst inferring a proposed new model for the lumbar region, anatomical differences were found in the cadaver used by the team from which they based their model data upon (Bayoglu *et al.*, 2017). For example, the Psoas major bundles were observed not at the previously documented lower L4/L5 disc (Bogduk and Macintosh, 1987) but at L1/L2 disc. This may have been affected by the L5 vertebra being fused to the sacrum, altering how the spine developed and moved (Figure 2). Whilst the focus of this thesis will be on the effect of axial loading on the IVDs and lumbar spine, the significance of the musculoskeletal components in both the functionality and stability of the spine in response to load/unloading is acknowledged. Changes in posture alone over time affect the fluid content of the IVD with greater fluid loss in a flexed/seated position compared to upright weight bearing (Adams and Hutton, 1983). Transitioning to movement requires both stability and balancing muscle movements through agonist-antagonist lines of action maintaining spinal control (Hsu, Castillo and Lieberman, 2015). Highly spindle dense small muscles connect to the processes of the lumbar vertebrae providing proprioceptive input and stability. These include the interspinales, which attach to the spinous process, the intertransversarii attaching to the transverse processes and the intertransversarii mediales, laterals and ventrales, which arise from the accessory and transverse processes, respectively (Adams *et al.*, 2013). The anterior abdominal muscles are made up of the rectus abdominis, obliquus internus and externus and transverse abdominis, with the psoas major positioned laterally alongside the vertebrae permitting the exertion of large forces upon the lumbar spine during hip flexion. Laterally posterior to the psoas major is the quadratus lumborum connecting between the 12th rib and the ilium providing structural support, particularly during lateral movement. Immediately posterior to the vertebrae is the multifidus connecting with the spinous process, with the longissimus thoracis and iliocostalis positioned laterally and connected to the accessory and transverse processes respectively. The extensors muscles in the back play a large role in mechanical stability and movement of the lumbar spine, whilst the anterior antagonist muscles, the abdominals are responsible for the sagittal flexion of the spine (Hsu, Castillo and Lieberman, 2015). Together with the vertebral processes/joints, IVDs and spinal geometry of the spine (i.e. the curvatures) these structures afford the spine its ability to defy axial and torsional stresses to facilitate locomotion and daily living on Earth.

Spinal curvature

In order to distribute loading in response to gravity, the vertebral column has evolved four main regional curvatures, the cervical (lordotic), thoracic (kyphotic), lumbar (lordotic) and sacral/coccygeal (kyphotic) (Figure 2). The kyphotic or anteriorly concave orientated regions develop initially at the embryo stage, with the thoracic curvature allowing the increase in width necessary for the thoracic cavities functionality. The lordotic or posteriorly concave orientated curves develop during gestation with foetal scans showing the presence of lordotic development as early as 23 weeks (Choufani *et al.*, 2009). The spine continues to be influenced through infantile development, incorporating functional movements in response to the progression towards upright posture. Gender difference of the spine also exists, with the upper lumbar positioned more dorsally in the female compared to male spines, presumably to reduce the stress on the vertebrae during pregnancy, though the degree of lordosis does not vary per se between genders (Hay *et al.*, 2015). Variation in the degree of spinal curvature does however exist between individuals. For example a high degree of kyphosis of the upper spine (or hunched back) can be attributed to several factors including ageing and disc degeneration (Ailon *et al.*, 2015). Lateral curvature is also present in the spine to a degree in most spines, though when it is in excess of 10^0 (as measured by the Cobb method) with accompanying vertebral rotation this is termed scoliosis which can severely hinder quality of life requiring interventions to straighten and support the spine (Goldberg *et al.*, 2008). The forces acting on the spine resulting from both the combined effects of gravity and demands for movement to operate in an upright position are not simply in one plane, otherwise the evolution of spinal curvatures would be superfluous. It is this rigid yet articulated curved structure that allows bipedal locomotion and the ability to traverse multiple environments, that can vary the degree of loading/demand on the spine.

Section 2.02 Investigating the effects of loading on the spine

<u>Overview</u>

Response to loading and unloading occurs at both the systemic and cellular levels. Cells are effected by the transmission of mechanical stresses and respond through mechanotransduction of these mechanical signals facilitating structural reorganisation of the cytoskeleton supported by adhesion attachment (Alenghat and Ingber, 2002). For example, fibroblasts change their production of proteins in microgravity such that collagen production decreases (Monici *et al.*, 2011), that has been observed in the decreased expression of collagen in microgravity cultured intervertebral discs (Jin *et al.*, 2013). In architecture, the use of tensegrity is employed to organise and stabilise a structure to carry a given load at a low cost of material by balancing tension and compression forces (Gilewski, Kłosowska and Obara, 2015). This is true too of the body at both the systemic and cellular level, with cellular shape reflecting an optimisation in the balance of mechanical stresses, whereby disruptions and maladaptation can lead to cellular degeneration and death (Chen *et al.*, 1997). Indeed tissues are not passive structures but are dynamic, responding to cues in the mechanical environment and modifying their composition and mechanical properties (Albrecht-Buehler, 1991; Klein-Nulend *et al.*, 1995). This adaptation to mechanical stresses is further observed in the systemic level of the IVD and spine.

As adults, we spend the majority of our time in either an axial loaded position (seated or standing) or unloaded (recumbent). These load/unloading phases are essential for spinal health, in particular the intervertebral discs, by inducing differing pressure gradients facilitating the movement/diffusion of fluid and essential nutrients in and out of the discs (Malko, Hutton and Fajman, 2002). Everyday postures including flexion and extension affect these properties by altering the stretch on the annulus of the disc by virtue of the tension generated by the trunk muscles, with flexed postures increasing the posterior stretch by 60% and decreasing the anterior by 35% (Adams and Hutton, 1982; Newell *et al.*, 2017). The degree of flexion/extension was measured in 208 volunteers by using sensors to record their lordosis angle, it was found that nearly 5h a day was spent, with the lumbar spine flexed between 20° and 30°, whilst only 24 minutes was found with the spin extended relative to the reference standing position (Rohlmann *et al.*, 2014). The situational demand places further stress upon the spine i.e. through movement based tasks and/or locomotion, where for instance rotational stresses affecting the torsion upon the discs and fibres, will also affect the stresses on the vertebrae, connecting tissues (ligaments, muscles) and IVD (though torsional stresses affect the disc to a lesser degree). For example lifting and carrying a weight in front of the body increases both the compressive and resultant force acting on the spine, shifting the centre of mass anteriorly putting further stress on the discs (Rohlmann, Pohl, *et al.*, 2014). Normal diurnal cycles result in swelling and compression of the IVDs, decreasing and increasing the hydrostatic pressure of the nucleus and stress on the annulus, thereby altering the disc height across the disc in proportion to the direction of the stresses. This alteration in load/unloading, facilitated by posture/gravity/situational factors has been observed in-vivo with one study observing an increase of 5.2mm in L1-L4 height following 8h sleep (Ledsome et al. 1996). The measurement of stature also informs the study of diurnal spinal height change (De Puky, 1935), which incorporates, primarily, influences from the spine but also from fluid compartments, for example compression of the heel pads (Foreman and Linge, 1989). Circadian changes of between 1.3 and 2cm have been reported in participants, corresponding to approximately 1% of total stature (Tyrrell, Reilly and Troup, 1985).

Use of non-imaging dependent modalities for assessment of load/unloading

Investigating the load/unloading effects upon the spine can be performed using several modalities. The measurement of stature through stadiometry as discussed has been used to quantify the effects of diurnal fluctuations, attributed primarily to spinal elongation (Tyrrell, Reilly and Troup, 1985) with the advantage of being low cost and portable and able to be utilised during standing, seated (Young and Rajulu, 2012) and supine postures (Dennis, Hunt and Budgeon, 2015). Clinically, supine height is recorded to infer drug dosage, ventilator support and nutrition and is recorded either with a metal tape, visual estimation (Bloomfield *et al.*, 2006) or through nomogram extrapolation of arm length (Todorovic, Russell and Elia, 2011). Seated height has been used to reduce the influence of the lower extremities on the measurement of spinal height (Rodacki *et al.*, 2001), whilst also facilitating the ergonomic assessments in wheelchair settings and spacecraft chair design (Brinckmann *et al.*, 1992; Young and Rajulu, 2012). However, being in a flexed seated position does change the spinal curvature, thus the distribution of forces across the lower spine.

In the assessment of curvature, a study compared the use of a flexicurve ruler against computer tomography (CT) for assessing thoracic curvature (Teixeira and Carvalho, 2007). These authors found a good agreement between flexicurve and CT measurements (interclass correlation coefficient or ICC:0.906). However, another found poor agreement between these methods (ICC:0.5; Azadinia et al. 2014) which could be attributed to study bias introduced in the first study as the authors took flexicurve measures in the CT scanner setup, prior to scanning. These low cost, portable methods are of utility in remote situations when imaging in not available and can also provide some insight into the effects of loading and unloading on height and curvature of the spine. However, these methods are only surrogate measures for studying the effects of load/unloading on the spine, for which imaging is required.

Use of imaging dependent modalities for assessment of load/unloading

Further detailed evaluation of the spine can be performed using imaging modalities including dual-energy x-ray absorptiometry (DEXA), CT and magnetic resonance imagining (MRI). MRI is favoured by some groups partly due the estimated effective radiation dose of a CT scan for a whole-body spinal scan, which can range from 11-20 milliSieverts (mSv) compared to 0 with MR. In relative terms this is a low radiation dose, with a risk for detriment to health calculated at 7.4% per 1000mSv (Fleischmann, 2010). In comparison to MR and CT, DEXA has primarily been employed to assess density changes in the body, including adipose tissue and bone density, however some groups have employed it to assess vertebral geometry (Humbert *et al.*, 2017) and intervertebral spaces which can provide some information on the disc heights (El Maghraoui & Roux 2008; Carvil et al. 2016). These modalities provide details on how the structures of the spine respond to load/unloading by assessing several criteria including IVD size (height, width, volume and bulging), spinal canal width, spinal length and curvature, muscle cross sectional area, vertebral and endplate integrity/shape, hydration (through spectroscopy), protein content (through contrast labelling), mechanical properties (through elastography) and pathological identification.

Due to the array of measures that can be employed, methodological and terminological differences can lead to some ambiguity in the literature (Van Tulder *et al.*, 1997; Fardon *et al.*, 2014). For instance, one study investigating how a 14-week special forces training schedule affected the lumbar spine, reported no impact upon the lumbar spinal structures with follow-up MRI (Aharony *et al.*, 2008). However, the authors fail to mention how the spine was interpreted/analysed, only that a radiographer assessed their spine, making any inferences into the effect of loading subjective. Before the development of upright MR scanners, in-vivo studies investigating the effect of loading had been done using supine MR and a compression frame. A custom-built harness which was MR compatible was made so that the participant could be loaded whilst in the MR, applying a percentage of bodyweight loading shoulder to foot (Willén *et al.*, 1997). A study using 50% bodyweight loading via a *Dynawell* MRI compatible harness, found a significant decrease in spinal length of 2.5mm and an increase/decrease in the intervertebral angle measures at L3/L4 and L5/S1 respectively. This was associated with the acute effects of loading, coinciding with other studies of this loading harness (Kimura *et al.*, 2000). Upright CT and MR can show alterations in the spinal structures in response to loading that otherwise can remain hidden, as well as alleviating anxiety from claustrophobia (Saifuddin, Blease and Macsweeney, 2003; Alyas, Connell and Saifuddin, 2008). For example upright MR found evidence of spinal canal deformations in several patients which was absent in a supine scan (Muto *et al.*, 2016).

An everyday loading task experienced by a wide range of the population from young to old is to wear a backpack, which has been studied with both upright and supine MRI. A study looking at this loading task in adults with upright MR, found a backpack with an extra 10% bodyweight loading induced a significant compression of the L4/L5 an L5/S1 anterior disc height, though only 6 participants were assessed (Shymon et al. 2014). Children often wear backpacks that due to their size are far greater in proportion to their bodyweight than a typical adult might utilise. Another study found that with increasing loads of 10, 20 and 30% bodyweight there was an increase in the disc compression in the midline of the lumbar spine and an increase in the lordosis of the lumbar spine compared to normal standing (Neuschwander et al. 2010). However, it is important to note these testing parameters are far beyond what is recommended by guidelines for children's backpacks of 10-15% bodyweight (Brackley and Stevenson, 2004). This level of loading has been observed to increase the rounding of the shoulders and forward head position with dynamic observations, which could affect the spinal column as a whole (Mo *et al.*, 2013).

Dynamic assessment, as performed with point to point positional imaging such as with supine vs. upright MR comparisons reveals data about how axial loading and posture effects the spinal structures. However, it cannot capture how the spine is moving over a range of motion, as flexion and extension alter the compressive and torsional stresses placed upon the spine, which can be further effected by the loading properties of the disc. Quantitative fluoroscopy is a technique that facilitates continuous assessment of inter-vertebral motion. Parameters can be derived including the maximal inter-vertebral range of motion during movement (Sayson *et al.*, 2015), how the motion is shared between the IVD levels (Breen and Breen, 2017) and how restrained, or how lax the disc is during motion (Breen et al. 2015). It's effective radiation dose of 0.561mSv is considerably smaller than a typical CT scan thus allowing multiple scans with different positions at a far reduced risk (Mellor et al. 2014). Ultrasound can also be used to assess the size of supporting spinal structures, IVD height, fluid dynamics and can be coupled with contrast agents to perform tissue characterisation. Whilst the imaging window and detail captured by ultrasound is confined, it is extremely portable and adaptable having been utilised on the international space station to demonstrate acquisition of lumbar and cervical disc height (Marshburn *et al.*, 2014a).

Other methods of spinal assessment

There are other modalities that can be employed with both static and dynamic assessments to assess the supporting muscles of the spine. These include but are not limited to myometry which uses a handheld portable device (Myoton) to study the viscoelastic response of the muscle by applying a brief mechanical impulse on the skin/muscle surface and measuring the oscillation feedback, to calculate muscle stiffness (Schneider *et al.*, 2015). Dynamometry and functional exercise tests can be performed to assess muscular strength (Demangel *et al.*, 2017), endurance and fatigue rate (Surakka *et al.*, 2001) and electromyography to analyse the electrical signals of the muscles to assess neuromuscular control (Jia and Nussbaum, 2016).

Finally, modelling for assessing the spine and its associated structures can provide critical information on the distribution of forces, stresses and dynamics which can inform suit ergonomics and design (Zhang, 2014; Kendrick, 2016), provide pilot data and inform current practices (Cholewicki and McGill, 1996). This data can be acquired from both patient scans and cadaver studies to inform computational models to explain the mechanical and musculoskeletal environment (Bayoglu *et al.*, 2017). This can assist in the explaining of the aetiology of several disc pathologies including disc herniation and degeneration (Robson Brown *et al.*, 2014; Zehra *et al.*, 2016).

Section 2.03 Disc herniation and degeneration

Overview

The IVDs have an inbuilt capacity for repair and renewal, however this capacity can be overwhelmed from disruptions in the mechanical environment. This can be observed through impeded nutrient flow and hydration affected via endplate damage, a build-up of minor tears in the IVDs, and abrupt trauma causing large scale tears in the annulus. In an update to the nomenclature and classification of lumbar disc pathology and recommendations from a combined task force of the North American Spine Society, the American Society of Spine Radiology and the American Society of Neuroradiology, the terminology and classifications of disc pathology were refreshed to provide a clear understanding across disciplines (Fardon *et al.*, 2014). A morphologically 'normal' disc is referred to when it is free of significant degenerative, developmental or adaptive changes relative to the clinical history of that person, as specific cases will influence the morphological definition of normal. Leading from this definition several categories emerge including congenial/developmental variations, degenerative, herniation, trauma, infections/inflammation, miscellaneous paradiscal masses of uncertain origin and morphologic variants of unknown significance. Whilst all these categories could have pertinence in any imaging study and or investigation such as those conducted in this thesis, for the purpose of clarity only disc herniation and disc degeneration will be discussed further.

Disc degeneration and herniation are not an uncommon finding on imaging scans as both can be asymptomatic without the participant aware of any underlying structural issues. In a study of over 26,000 lumbar discs taken from lumbar MRI scans of 5000 patients over 2 years, the study found hernias in 14% of discs and degeneration in 44%, with the lower lumbar discs L5/L5 and L5/S1 having the highest prevalence (Zhang *et al.*, 2016). However, no data on the incidence of lower back pain was recorded or correlated.

Disc herniation

Disc herniation occurs when the disc is not able to resist the forces placed upon it, which can be due to existing damage i.e. scar/fissures, decreasing resistance and/or the application of combined forces i.e. compression and torque (Marshall and McGill, 2010) resulting in high incidence of herniation. Disc herniation is broadly classified as the localised displacement of material beyond the normal confines of the intervertebral space. Disc material can be displaced beyond the 'normal remit' of the ring apophyses both symmetrically or asymmetrically and still be classified as a bulge, not a herniation (Fardon *et al.*, 2014). Identification of a bulge can be observed by measuring outward from the middle of the disc, the guidelines suggest that approximately a 25% circumferential (either symmetrical or asymmetrical) expansion is considered a bulge, though the precise measurement can very between groups leading to methodological difference thereby affecting radiographic interpretation and inferences (Van Tulder *et al.*, 1997). For example, one study used a bulge size of <3.2mm as a criteria for bulge identification (Luoma *et al.*, 2000) with the authors observing an association between reports in the preceding year of lower back pain and findings of disc bulging. Another using T2 weighted MRI sagittal scans of the lumbar spine reported high T2 visuals (corresponding to an increase in the brightness) in the posterior 10% of the annulus fibrosis, which indicated the prevalence of a bulge where changes in the posterior 20% of the annulus related more to changes in the nucleus pulposus, indicating a herniation (Messner *et al.*, 2017). Another study classed a bulge as any visible posterior displacement of the IVD over the boundaries between the adjacent vertebral bodies, with a herniation described as protrusion or extrusion of nucleus material outside the confines of the annulus (Cheung and Karppinen, 2016).

Whilst the terms protrusion and extrusion are used their meaning can vary between sources. Protrusion is associated with a localised protrusion of the outer annulus containing nucleus material within, due to rupture of the inner annulus. Extrusion is when the inner and outer annulus have been compromised and nucleus material (amongst other material including collagen and endplate fragments) is displaced through a fissure outside of the disc (Adams *et al.*, 2013; Fardon *et al.*, 2014). The terminological difference employed in studies can lead to difficulties comparing study findings and the potential for differences in treatment outcome and perscription.

Treatment pathways

With treatment paradigms patient history, contraindications, desired outcomes and current physiological and mental wellbeing health ultimately play a role in deciding the optimal treatment pathway. Surgery is a treatment pathway to manage symptomatic disc damage and abnormalities, utilising techniques including microdisectomy, endoscopic microdiscectomy, transforaminal endoscopic discectomy and laminectomy with discectomy, each with its own success stories and limitations. A recent study on the long term outcomes of surgery from literature found out of nearly 40,000 patients 79% reported good to excellent results (Dohrmann and Mansour, 2015). A randomised controlled trial comparing groups who underwent transforaminal endoscopic discectomy vs. microdisectomy highlighted that microdectomy resulted in a less frequent revision rate (re-admittance), but in a longer recovery time due to damage to the surrounding musculature (Gibson, Subramanian and Scott, 2017). Surgical procedures ablating the annulus could cause large scale structural damage to the outer annulus, that will affect the collagen structure and be replaced by scar tissue thereby reducing resistance to imposed stresses, which could lead to further degeneration (Shankar, Scarlett and Abram, 2009). Alternately, there are a plethora of other non-surgical methods which have been studied including exercise prescription, physical therapy, psychological counselling, injection and medications (Saal, 1996). However, there are a multitude of factors to consider not only for treatment but also in terms of monitoring and outcomes (Awad and Moskovich, 2006). As such it is important to consider this when assessing the effectiveness of treatments. For example, one study investigated if the measurements taken from MRI at baseline and follow-up in a group of patients diagnosed with lumbar disc herniation, could correlate with other clinical outcomes including questionnaires, visual analogue scales of pain and degree of spinal movement (Kamanli et al. 2010). Using a combination of spinal traction, physical therapy and ultrasound on 26 patients they found a significant decrease in the pain and movement restriction. However from the MRI scans five patients decreased the degree of bulging measured, three increased and the rest observed no change (Kamanli *et al.*, 2010). The authors state that spinal traction was effective in treatment of subacute disc herniation, which considering the multitude of interventions in their study could be construed as a misleading statement. However, the authors also state that structural findings do not correlate with subjective incidence which has been shown in other studies (Borenstein *et al.*, 2001). In a systematic review of the literature which graded a number of studies in terms of quality, it was concluded from the 18 studies selected, an association between disc degeneration with non-specific lower back pain exists but due to the methodological difference between studies no causal relationship can be made (Van Tulder *et al.*, 1997). The common element is that disruption in the mechanical environment and/or incidence of increased structural injury (i.e. potentially caused by intervention) can lead to further degeneration (Ruan *et al.*, 2007).

Disc degeneration

Degeneration occurs in the IVDs with a host of potential contributing factors including a reduction in the nutrition of the nucleus pulposus, that can be brought on through ageing and/or structural damage induced through stress overload (Buckwalter, 1995). Using an imaging technique called phase contrast synchrotron micro-tomography assessment on the IVD and endplates, a study in young and old mice, found that in older mice there is a decrease in the endplate porosity and thickness as well as the density of connecting nutrient canals (Cao *et al.*, 2017). The change in the density of the connecting canals could be an attributing factor to the reduction in nutrient supply to the disc (leading to degeneration) as a study in human IVDs found endplate density and thickness to be independent of age (Wang *et al.*, 2011). Indeed larger defects in the endplate which could thereby affect metabolite transport have an association with disc degeneration and reduction in IVD volume and decreased intradiscal pressure (Zehra *et al.*, 2016). This affect upon the IVDs and the nucleus could thus impact the matrix of the nucleus affecting the regulatory factors expressed in this environment, thereby inhibiting the regenerative capacity of the region (Liu *et al.*, 2015).

In a study of 300 lumbar specimens from a spectrum of ages, individual signs of degeneration were seen as early as the 2^{nd} decade of life as determined by Nachemson's 1-4 grading scale (Nachemson, 1960), with an increased prevalence in adult males (Ashton-Miller, Schmatz and Schultz, 1988). The Modic scale is a 3 point scale used to characterise changes in the vertebral marrow (and endplates either side of the IVD) corresponding to a change in the signal intensity of an MR scan; a type 1 Modic change is described as a sign of endplate fissuring with vascular infiltration, a type 2 Modic change with an increase in granulated tissue in the endplates and fat infiltration in the body and a type 3 as sclerosis of the bone (Modic *et al.*, 1988; Albert and Manniche, 2007). A relationship between disc herniation and development of Modic changes (type 1 predominantly) has been observed in several studies (Mitra, Cassar-Pullicino and Mccall, 2004), with an extension of Modic type 1 changes strongly associated with a worsening of patient's symptoms of lower back pain (Albert & Manniche 2007). However structural changes do not necessarily translate into an experience and/or change of symptoms (Teichtahl *et al.*, 2016). Another scale used in classifying disc degeneration is the 5 point Pfirrmann scale, which takes several factors including structure, distinction and appearance of the nucleus pulposus, disc height and the signal intensity to provide a grade of degeneration, with a grade of 4-5 corresponding to severe disc degeneration (Pfirrmann *et al.*, 2001). A study investigating the relationship between Modic changes (signal intensity) in the endplates and degeneration in the IVDs found there was an association between degeneration in the disc and its adjoining structures including an increase in adipocyte content of the muscles that appears to accumulate in the perimysal spaces (Teichtahl *et al.*, 2016). This could reflect an affect upon the nutrition transport both towards and away from the disc and surrounding tissue, or perhaps an environment favouring an adipocyte trans-differentiation pathway of the fibroblasts (Agley *et al.*, 2013). In an experiment in mice investigating the utility of stem cell therapy for preserving muscle loss under extreme disuse conditions (i.e. long duration spaceflight), the authors found an increased preservation of muscle in the leg which was injected with stem cells that had been pre-cultured for a microgravity setting (Ohi *et al.*, 2004). The inverse of this has been observed in other tissues, such as myocardial tissue where an increased workload on the heart in response to exercise, induces vascular remodelling via the stem cells (Waring *et al.*, 2014). A US clinical trial (https://clinicaltrials.gov/ct2/show/NCT02412735) is currently recruiting to determine the effects of stem cell replenishment as a treatment method for symptomatic disc degeneration patients (those experiencing back pain). One thing for these therapies to consider is how the mechanical environment upon the disc and it's 'workload', could be optimised to compliment treatment.

An imbalance in the mechanical environment/stimuli of the disc could also affect the expression of regulatory factors in the surrounding tissues (Ma *et al.*, 2015), which may further influence the differentiation of the progenitor cells (stem cells) leading to degenerative changes. Disruption in the mechanical environment is also thought to be a contributing factor to chronic, non-specific, lower back pain, with an accompanied alteration in the intervertebral kinematics observed during passive motion (Breen and Breen, 2017). A population study looking at combined degenerative changes of the disc and the endplates (through Modic signal changes using MRI) found an association with reporting of lower back pain (Teraguchi *et al.*, 2015). Other studies have also found an association between incidence of lower back pain and lumbar disc degeneration, which increases in the severity of disc degeneration (Cheung *et al.*, 2009), suggesting a structural, mechanical element to back pain. However, this is not indicative of causality as not all disc degeneration leads to back pain, but rather an association.

Back pain is a multifaceted condition, that can incorporate psychological, neurological, somatic and nociceptive inputs (Flor, 2002). It can be assessed through several techniques including visual analogue scales (Treffel *et al.*, 2017) and questionnaires such as the Oswestry disability index (Davidson and Keating, 2002). Prolonged unloading of the disc through a change in the mechanical stimulus can be accompanied by back pain, such as with bedrest (Hutchinson *et al.*, 1995) and spaceflight (Wing *et al.*, 1991). During a 3-day study where individuals floated in a barrier protected water tank, termed dry immersion, disc swelling was observed using IVD volume analysis and spectroscopy via MR, with 92% of participants also reporting back pain via a 1-10 visual analogue scale (Treffel *et al.*, 2016, 2017). However, due to low subject numbers (n=11) a relationship between imaging changes and pain development could not be appropriately determined. An investigation into patients with chronic lower back pain who underwent disc surgery due to nerve root compression, sought to quantify their clinical symptom progression using imaging (diffusion tensor imaging) in tandem with questionnaires (Oswestry disability index) (Wu *et al.*, 2017). They found a strong correlation between imaging and questionnaire measures, preliminary suggesting it might be possible to use this technique to evaluate and follow-up the clinical progression post disc surgery. However, while imaging advances are permitting further investigation into the links between back pain and disc abnormalities, there is still no agreed reliable clinical diagnostic tool to determine if the disc is the source of back pain (Brayda-Bruno *et al.*, 2014). Physiologically, it is not only the mechanical but also biochemical changes that require investigation and consideration.

Lastly, genetic variations are also a contributing factor to consider in the development of disc degenerations (Battié, Videman and Parent, 2004). In a recent study integrating imaging and genotyping data with computational modelling, the authors found a number of single nucleotide variants coding to the proteoglycan aggrecan that were associated with the degree of lumbar disc degeneration in patients with chronic lower back pain (Perera *et al.*, 2017). The combination of genetic influences and ageing could explain why in high loading/risk activities such as aviation (Mason, Harper and Shannon, 1996) some individuals enjoy greater protection. Also it could be why in studies, occupation is observed at a contributing factor towards the development of disc degeneration (Luoma *et al.*, 2000). From the culmination of all these contributing factors several definitions of disc degeneration can be provided, however perhaps one of the most pertinent to this thesis is ‘a sluggish adaptation to gravity loading followed by obstructed healing’ (Lotz, 2004; Adams *et al.*, 2013). Therefore, situations where there is a chronic absence of gravity has unsurprisingly profound consequences.

Section 2.04 Spaceflight and the spine

Overview of the effects of spaceflight on stature elongation

Microgravity experienced in space, induces a plethora of changes in the human body (Williams *et al.*, 2009), in particular the spine where a 1-3% stature elongation is reported (Stoycos and Klute, 1993). One astronaut, Scott Paralyski, during his shuttle missions (the longest being 16 days), experienced stature elongation of 5.1-5.7cm compared to his normal height on Earth (Sayson *et al.*, 2013a). Furthermore, during an early Apollo mission a “two stage” elongation was observed, with an average of 1.3cm within the first 6 days of flight and 3.9-6.9cm during days 8-9 whilst, in the Apollo-Sozuz test project mission (ASTP), they reported an elongation of 2.5cm in the first 6 days of flight (Nicogossian, 1977). In longer duration Skylab missions, after 21 days in space there was an average of 4.7cm elongation vs. pre-flight, increasing to 6.2cm after 80 days in space (Thornton, Hoffler and Rummel, 1977). These initial findings suggest that a plateau exists after an initial increase in stature in the early mission phase (Thornton, Hoffler and Rummel, 1977). It is important to note from these early missions the ambiguity with the recordings of stature. Firstly, in the limited number of crewmembers measured, despite the large number of individuals who have now gone to space (estimated at near 550 upon this thesis submission). Secondly, in the measurement of stature, which has involved placing the crewmember against the wall, marking the position of the head/foot and measuring between them (Thornton, Hoffler and Rummel, 1977). A recent NASA study has sought to provide clearer data using a fixed video camera system with reflective markers to provide more depth of data and reduce human error. The study is ongoing but has so far tested 5 out of a proposed 8 astronauts, reporting 1-3% total stature elongation, this follows the same trend as previous studies where an initial large elongation is reported that appears to plateau during the mission (Sudhakar *et al.*, 2015).

<u>Operational issues with stature elongation</u>

Operationally stature elongation presents issues for the donning of spacesuits, with early spaceflight operations requiring colleagues to physically compress them into their spacesuits. Sub-sequential suits were created with an additional 2.5cm margin. Though issues are still experienced in space as well as fitting into re-entry vehicle seats on the Souyuz, which are custom made pre-flight to minimise the transmission of G turbulence on the body (Thorton & Moore, 1987; Nicogossian, 1989). Thus further studies have deduced that space suits must provide enough adjustability to allow for the elongation of the human spine in microgravity of up to 3% total stature (Rajulu and Benson, 2009). Another consideration is craft design and mission parameters. With the future NASA Orion vehicle, measurements of seated elongation might be more critical to consider than total stature, for capsule and seat design. A study evaluating differences in both seated and total stature elongation reported that up to 6% seated elongation, corresponding to 3% total stature elongation, must be considered when designing compartment layout (Young and Rajulu, 2012). Whilst this is important for spaceflight ergonomics, it is the resultant issues attributed to elongation (and the loss of axial loading on the spine) that are more pertinent for space agencies (Belavy *et al.*, 2016).

Back pain in space

Back pain is a common issue in spaceflight with over 68% of crew members in one study reporting acute lower back pain (Wing *et al.*, 1991). While the precise pathophysiology is not known, it is currently attributed to super-normative disc expansion and deformation, reduction in hydrostatic pressure and soft tissue stretching (Sayson and Hargens, 2008). As a result astronauts typically adopt a "fetal tuck" position in space, documented since early space missions (Thornton, Hoffler and Rummel, 1977) to provide relief from back pain, potentially through increasing the force on the disc, generated through flexion, thus inducing compression (Sayson *et al.*, 2013). A theory of why back pain reduces is a reduction in disc height/volume, as there is a stretch of the collagen structures of the surrounding ligaments and joint capsules, stimulating type 1 and 2 mechanoreceptors, which could alter the opioid balance, neutralising the build-up of pain inducing neurotransmitters (e.g. substance P), thus providing an analgesic effect (Korr, 1986). A project is currently ongoing to provide greater detail into in-flight back pain (Snijders *et al.*, 2009).

Spinal muscle and skeletal effects of spaceflight

In a study of musculoskeletal injuries in NASA astronauts in-flight, minor back injury was the second most reported injury (below hand injuries) in particular in relation to exercise (Scheuring *et al.*, 2009). In an imaging study comparing pre-and post-flight changes in the spine of six NASA crewmembers, a post-flight 14% reduction in paraspinal lean muscle mass was observed taken from an average of all the paraspinal muscles, using functional cross sectional area at the L3/L4 level which was chosen for ease of defined muscle boundaries (Chang *et al.*, 2016). However, another study on a single ESA astronaut found that the measured size (using ultrasound) of the multifidus was maintained post-flight (attributed to exercise countermeasures) at the L2-L4 level but at L5 was reduced, with an accompanying reduction in transverse abdominus size (Hides *et al.*, 2016). A strength of the NASA study was the increased N but unfortunately unlike the ESA study the components of the paraspinal muscles were not broken down, but summed and only done at L3/L4. On Earth a decreased size of the paraspinal muscles is linked to an increased fat infiltration and muscle degeneration (Kalichman, Carmeli and Been, 2017) which could predispose this population to further injury post-flight. A study comparing healthy controls with a group suffering from degenerative spinal stenosis noted an increase compared with healthy controls in the density and size of the paraspinal muscles (erector spinae and psoas) which was linked to lower back pain. However this was measured at the L2/L3 level (Abbas *et al.*, 2016) and may indicate that it is the lower levels at L5 which are more susceptible to degeneration and should be investigated further despite imaging difficulties (Chang *et al.*, 2016). How this relates to mitigating the risk of injury and informing both exercise countermeasures and post flight rehabilitation is an area of ongoing work, as both high and low back loading on return effect the motor control of the lumbar spinal muscles differently (Callaghan and McGill, 1995). If compromised further, especially with low loading tasks (i.e. picking up a pencil) this could be a contributing factor to this population's increased risk of disc herniation.

<u>Disc herniation in astronauts</u>

The astronaut population has one of the highest incidences of disc herniation. (Johnston *et al.*, 2010). A NASA study comparing the astronaut population against a control sample of NASA employees found the risk of a herniated nucleus pulposus (HNP) occurring was 4.3 times higher in the astronaut population compared with control, with 44 cases reported, 22 in the lumbar and 18 in the cervical region. Though less frequent, there is a documented increase in the reporting of HNP in army aviators, the cause of which remains unknown (Mason *et al,* 1996). Out of 132 reports of HNP over 5 years (1987-1992), 25.8% had cervical HNP and 74.2% had lumbar HNP (no thoracic were documented). A point to consider here is that the detail from the scans in lacking. The term HNP implies the nucleus is herniated where a herniation could be made up of other disc constituents. A cadaver study investigating the effect of stress test failure found the annulus fibres (which attach to the end plate) stripped elements of the hyaline endplate from the bone, thus this could explain the appearance of other disc material in herniated discs not just the pulposus (Balkovec *et al.*, 2015). Future studies should seek to clarify these expressions. Whilst some studies have shown signs of recovery of the IVDs within a few days of ambulation following a 5 weeks bedrest trial, it took up to 6 weeks to recover in those who underwent 17 weeks bed rest (LeBlanc *et al.*, 1994) and up to two years in another more recent 60-day bed rest trial (Kordi *et al.*, 2015). These differences between studies could be due to advances in both modality and sensitivity of imaging to detect alterations in the IVDs. Using upright MRI and quantitative fluoroscopy, observations in one astronaut saw larger IVD heights in the lumbar spine on return to Earth vs. Pre-flight, as well as reduced flexibility, associated with a chronic over-saturation of the IVD (Chang *et al.*, 2014). There was also reduced amounts of proteoglycans, an associated factor with disc degeneration on Earth (Lyons, Eisenstein and Sweet, 1981). Whilst research exists linking lumbar HNP to IVD swelling, deformation and reduced proteoglycan content, more knowledge of the effects on load/unloading on the lumbar and the cervical spine is needed (Belavy *et al.*, 2016). As such analogues are required to further the understanding of how the spine responds to load/unloading and to evaluate potential countermeasures for spaceflight.

Whilst the focus of this PhD will be on the change in the load/unloading paradigm induced by microgravity, it is important to recognise the effect of cosmic radiation and how this can further induce deleterious effects including DNA strand breaking, mutations and apoptosis that could further affect cellular repair and renewal (Fang, Yang and Wu, 2002).

Section 2.05 Analogues of microgravity

<u>Head down Tilt</u>

Studying microgravity on Earth is performed with a range of analogues. The most common analogue is head-down tilt (HDT) (Pavy-Le Traon *et al.*, 2007). A subject is positioned onto a table/bed and rotated around the central axis either up or down thereby facilitating fluid shifts, and load/unloading the spine, with forces transferred feet to head, rather than head to feet (Figure 3). Supine bed rest has been studied though a 32-hour bed rest failing to see a significant increase in elongation greater than 8 hour sleep (McGill and Axler, 1996). The length of stay during HDT studies varies from a few hours to several months, with longer term HDT (>7days) utilised to investigate the effects of musculoskeletal atrophy on the body (Hargens and Vico, 2016). 6^{o} HDT is the typical angle used to simulate spaceflight (Styf *et al.*, 1997) and has been employed to study the effects of unloading on the spine and its associated structures (Belavý et al. 2010). After 56 days of HDT it was found that after 2 years the lumbar IVD had still not fully recovered with an increase in IVD volume, height and length (Belavý, Armbrecht and Felsenberg, 2012). Another study on 21 day HDT found an unexpected result immediately after bedrest (Koy *et al.*, 2014). The authors reported a decrease in the T1 signal intensity, which by using a contrast agent was associated with an increase in glycosaminoglycan content. This is counterintuitive to what is reported with unloading from spaceflight (Sayson *et al.*, 2015). This may be due to the hydrostatic gradient induced by tilting during HDT which is not the same as spaceflight. It was also documented that hypertrophy of the cervical muscles and thoracic discs occurred with prolonged HDT (Belavý *et al.*, 2013). These findings may indicate that as the individual is tilted downwards (albeit slightly) this results in an axial vector cranially and/or this tilted position precipitates greater demand on the neck musculature to facilitate participant wellbeing. Back pain, another symptom in microgravity has been reported with HDT commencing in the early phases before reaching a plateau and dissipating when loading on the spine resumed (Hutchinson *et al.*, 1995). A recent review of concluded that HDT does not provide a suitable platform to simulate the fluid shifts experienced in space, nor the unloading effects observed upon the spine. This is due to cranial tilting and the imposition of a G vector, however a supine posture which induces an even hydrostatic gradient is proposed to be more representative of the spaceflight environment (Hargens and Vico, 2016).

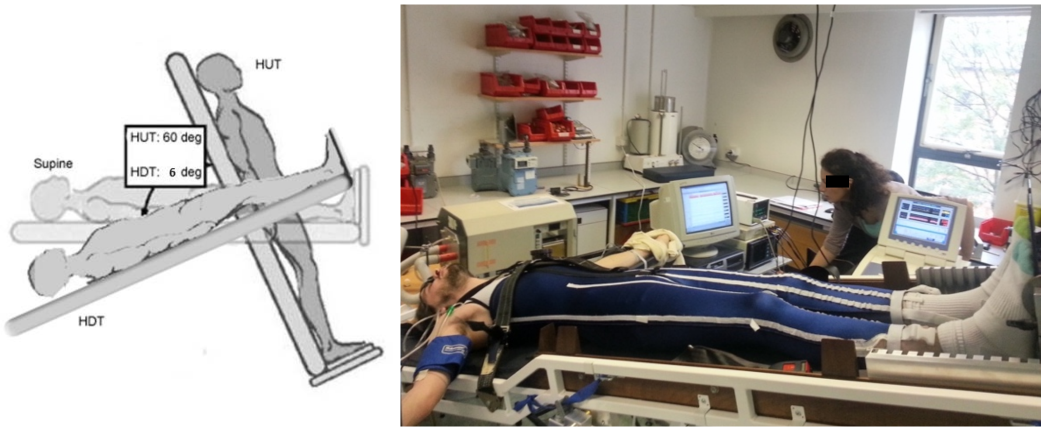


Figure 3. Diagram of tilting positions including head-down tilt (left) and participant in early SkinSuit design being tilted 6^{o} head-down to assess hemodynamic changes (right). Image credit King's College London.

<u>Spinal Traction</u>

Traction is an old method whereby a force, that can be imparted through manual, mechanical or inversion, pulls on the spine to alleviate symptoms induced by a reduction in the intervertebral spaces and supporting structures (Mathews and Hickling, 1975). Monitoring of the intradiscal pressure in the L4/L5 IVD whilst applying a traction over multiple sessions resulted in an average reduction of 100mmHg, which the authors attributed to be a potential mechanism for the alleviation of symptoms through improving the mechanical environmental (Ramos and Martin, 1994). To date though the use for or against spinal traction in clinical guidelines for treatment of lumbar disc herniation with radiculopathy is still undecided (NASS, 2012). It has been reported to increase stature temporarily by up to 0.5-0.7cm (Rodacki *et al.*, 2007) and has also been combined with HDT studies (Styf *et al.*, 1997), though as discussed this does not unload the body in the same manner as spaceflight or when buoyant.

<u>Water Immersion</u>

Direct water immersion has been used as an analogue to evaluate potential spaceflight countermeasures in the past (Barer *et al.*, 1972). In water immersion Archimedes' principle states that the upward buoyancy force acting upon the object is proportional to the volume of fluid displaced, due to this effect immersion is often used in rehabilitation and training settings (Torres-Ronda and Del Alcázar, 2014), though this typically require large bodies of water. Also, due to the direct contact with the water participants cannot stay in the tank too long, thus a barrier would be needed to facilitate long term assessments (Barr, Clement and Norsk, 2015). As such it is more often used for the extra-vehicular vehicle (EVA) training of astronauts in large neutral buoyancy facilities.

Dry Immersion

An alternative model to study spaceflight is dry immersion, where a body of contained water, covered with a non-porous sheet, facilitates the buoyancy of the subject allowing their feet and body to sink into the water (Figure 4; (Navasiolava *et al.*, 2011)). However, as it uses normal water as the medium, the body sinks into the tank thus creating an axial load ventrally down the spine towards the hips, which are flexed due to the proportional mass unloading (Navasiolava *et al.*, 2011), this also does not represent the hydrostatic gradient of space as there is a Gz vector. Also due to the differences in density the person sinks and the water envelops the individual, this can reduce pulmonary function due to the pressure of the water upon the thorax (Andrade Dornelas *et al.*, 2014) and has been cited as being uncomfortable for the participants deep breathing (Navasiolava *et al.*, 2011). However, this analogue has been effective at reporting rapid effects upon the lumbar spine. After a 3-day immersion period, there was a significant increase in lumbar IVD water content as measured by MRI spectroscopy by 17%, with an increase in IVD volume of 8-9% in the T12/L1 and L5/S1 IVDs. Similar to the 3-day HDT study (Hutchinson *et al.*, 1995), participants reported back pain identified as just below the diaphragm. However, it is important to note that the enveloping pressure of the water could be a contributing factor. Due to volume of water to maintain thermo-neutral, heathers, blankets and barriers are required to try to warm the subject, potentially leading to skin irritation and sores as they are enveloped. Whilst measurements can be taken pre-and post-flotation, monitoring the participant elongation with portable techniques like stature or ultrasound cannot be undertaken due to accessibility restraints. Thus, a model where the participant does not sink into the medium and is buoyant would facilitate improved accessibility.

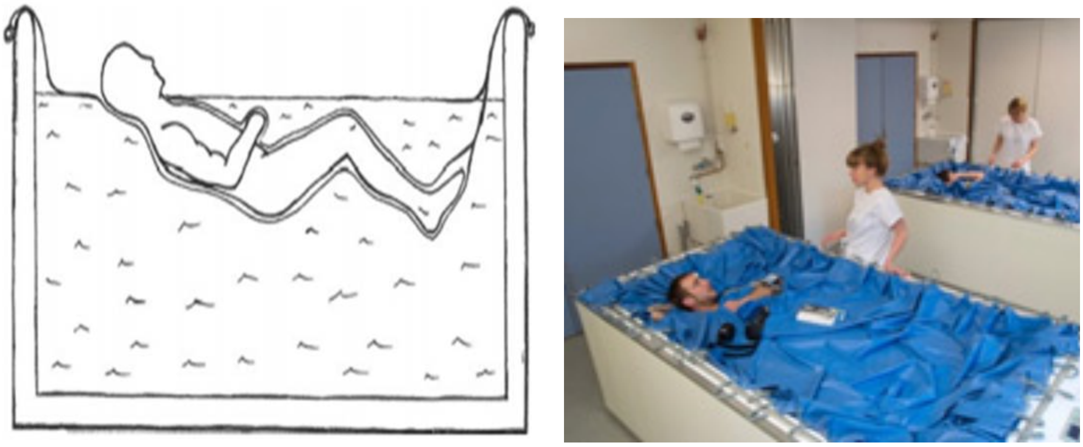


Figure 4. Representation of dry immersion reproduced from Navasiolava et al, 2011 (left – image credit) and photo of a participant in a dry immersion tank at the French research institute MEDES (right- image credit).

Restricted environmental stimulation technology flotation and the development of Hyper-Buoyancy Flotation (HBF)

The Dead Sea, due to its high saline content is often photographed with individuals floating upon the surface due to the density of the water, where the person is buoyant whilst resting upon the surface of the water. Originally conceived by John Lilly in the 1970's, immersion tanks filled with salt water, termed restricted environmental stimulation technology (REST) flotation has been used in therapeutic situations as a relaxation tool. Participants are placed in a darkened tank filled with mixed water and Epsom salt (Magnesium sulphate; 1.7gcm^3) which increases the density of the water in a manner analogous to the Dead Sea, thereby inducing a hyper-buoyant state of flotation (Hill *et al.*, 1999). Using a combination of principles from dry immersion and REST, a new analogue was devised, termed hyper-buoyancy flotation (HBF). HBF will be detailed further in Chapter 3, but briefly it comprises of a waterbed part filled with hypersaline water a to facilitate a supine, buoyant state, that is separate from the water and provides direct access to the participant for spinal assessments (Carvil *et al.*, 2015).

Section 2.06 Countermeasures for human spaceflight

Overview

Countermeasures are vital to maintaining astronaut health both physical and mental, with a focus on maintaining adequate stimulation of the musculoskeletal and cardiovascular systems due to the reduction in 1G to microgravity. A wide spectrum of countermeasures are thus employed (or have been) including pharmacological, lower body negative pressure, bungee cords, loading suits, nutritional optimisation/loading and exercise (Kozlovskaya and Grigoriev, 2004; Kozlovskaya *et al.*, 2006; Petersen *et al.*, 2016). The benefits of exercise both on Earth and in the space programs is well known, able to positively impacting the heart and wider cardiovascular system (Moore *et al.*, 2010; Waring *et al.*, 2014), bone density and musculoskeletal strength (Shackelford *et al.*, 2004; Kleinberg *et al.*, 2016) and the spine and its associated muscles (Kibler, Press and Sciascia, 2006; Sasaki *et al.*, 2012; Holt *et al.*, 2016). As such a host of exercise modalities have been employed on the ISS, with astronauts typically exercising for up to 2h per day (Williams *et al.*, 2009) to induce an increase in workload and thus adaptation in these physiological systems. The following section will make use of their acronyms of exercise modalities used in space. For reference please refer to the following paper and/or the glossary of abbreviations (Petersen *et al.*, 2016).

<u>Exercise Countermeasures</u>

At this point it is important to understand that these exercise modalities can act to load the spine. Cycling is a low volume and relatively easy countermeasure to perform in contrast to other modalities. It is achieved by anchoring the astronauts' feet to the stirrups using clips on an upright cycle (CEVIS) providing up to 250W. Work of loading (not power output) can be modified on the Russian cycle ergometer with motor driven bungee cords to impart greater loading on the foot crank in a resistance manner up to 30kg (VELO). Further resistance exercise including movements where the action is done axially shoulder to foot including heel lifts, propulsive jumps and squats can be done using a piston driven pneumatic exercise device imparting loads between 2.2-272kg (ARED). Finally running can also be achieved, affecting both the cardiovascular but also the loading imparted on the spine via a treadmill (COLBERT; that can be switched from active to passive modes). A harness which was recently upgraded uses bungee cords to impart loading on the treadmill up to 100% bodyweight with improved comfort (Figure 5) (Genc *et al.*, 2010; Petersen *et al.*, 2016). The treadmill is designed to load to full bodyweight of 100%, in reality the crew members start with the recommended 50% bodyweight then increase during the mid-phase of the mission to 70% (Petersen *et* *al.*, 2016). Subjective accounts from this author whilst trialling this harness on a treadmill whilst running at 70% bodyweight (albeit whilst on earth's 1G) proved uncomfortable to maintain for a long duration with a perceived, considerable axial compression. A further subjective account from an astronaut who measured themselves pre-and post-loaded treadmill exercise on the ISS reported that their stature reduced after running to near his Earth 'norm'. Whilst this lacks scientific rigour, it is to highlight that astronauts undergo acute, high bouts of loading upon the spine regularly, but still present with an increased risk of herniation (Johnston *et al.*, 2010) and atrophy of the lumbar paraspinal muscles (Chang *et al.*, 2016).

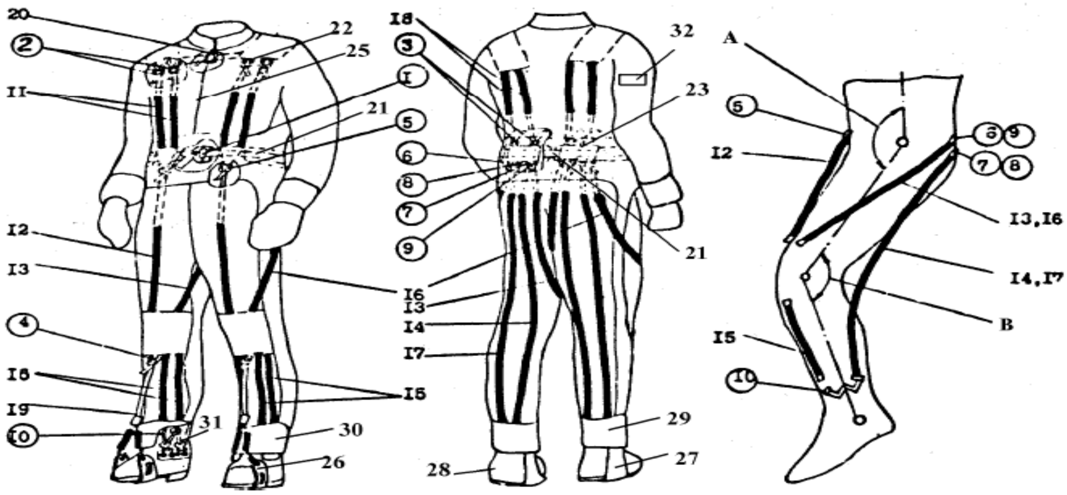
Figure 5. Exercise Countermeasures currently on use on the International Space Station including cycling on CEVIS (top left), resistance exercise on ARED (bottom left) and treadmill running on COLBERT (right). Image Credit ESA and NASA.

These acute bouts of high loading, without the prior torsional stressors on the spinal structures facilitated by the active resistance against gravity, could be a contributing factor for the aforementioned high prevalence of back injuries in space (Jennings and Bagian, 1996; Scheuring *et al.*, 2009; Somers, Gernhardt and Newby, 2015). Therefore, to date acute daily exercise countermeasures do not fully protect astronauts against microgravity-induced physiological de-conditioning (Payne, Williams and Trudel, 2007), in particular the IVDs and the supporting muscles (Chang *et al.*, 2016). As such a method of imparting a stimulus like gravity over time, might afford greater protection in-flight, especially when combined with existing countermeasures.

Imposition of artificial gravity

'Artificial gravity' and Gz vectors for extended durations can be provided via centrifugation, though issues do arise. With small centrifuges due to the increase in rotation around a smaller arm, the vestibular system is impacted by the Coriolis forces, this can be mitigated with a suitably long axis of rotation. However such a large centrifuge for humans requires considerable technological and engineering capability currently unavailable (Lackner and DiZio, 2000; Duda, Jarchow and Young, 2012). As described exercise countermeasures impart a Gz vector proportional to % bodyweight reported at the foot for acute periods of time relying on pneumatics and elastic materials. Loading suits have used elastic materials to impart loading to the body in space and have been used in the Russian program since the early years of spaceflight.

The TNK V-1 Pingvin suit created by Arnold Barer, utilises bungee cords running from the shoulder to a waist belt and the feet to provide ~70% static axial loading during treadmill running (Figure 6) (Kozlovskaya and Grigoriev, 2004; Barer, 2008). Cosmonauts that adhered to integrated suit and treadmill exercise experienced attenuated lumbar vertebrae bone mineral density loss (0-3%), compared to non-adherer's (6-10%) (Kozlovskaya, Grigoriev and Stepantzov, 1995). Whilst in a bed rest study of four participants, those that wore the suit ten hours a day preserved their Soleus muscle volume (Ohira *et al.*, 1999). A conference report on an operational assessment of the suit compared a suited group of participants against an unsuited group (5 v 5) after five days of water immersion (Barer *et al.*, 1972). It was found that wearing the suit, increased recovery from orthostatic intolerance, preserved or increased bone density (as opposed to a 4-8% loss in the other group) and what is termed a positive trend to improve the postural muscles of the back, though precise assessment techniques are lacking in the report. In contrast to the positive effects it has been purported to induce significant thermal and movement discomfort to the point that a number of cosmonauts did not adhere to suit utilisation. Such discomfort may be attributable to the two-stage loading design which does not replicate Earth's gravity (Waldie, 2005). It should be noted that the Pingvin suit is no longer in use.



Pingvin-3 constant-loading suit: 1 – Belt adjustment buckle; 2 – Chest tension strap adjustment buckles; 3 – Back tension strap adjustment buckles; 4 – Stirrup strap adjustment buckles; 5 – Front leg tension strap adjustment buckles; 6 – Slanted outer back leg tension strap adjustment buckles; 7 – Straight inner back leg tension strap adjustment buckle; 8 – Straight outer back leg tension strap adjustment buckles; 9 – Slanted inner back leg tension strap adjustment buckles; 10 – Height adjustment buckles; 11 – Chest tension straps; 12 – Front leg tension straps; 13 – Slanted inner back leg tension straps; 14 – Straight inner back leg tension straps; 15 – Uniformly tensioned leg tension straps; 16 – Slanted outer back leg tension straps; 17 – Straight outer back leg tension straps; 18 – Back tension straps; 19 – Stirrup strap; 20 – Horizontal chest strap; 21 – Belt; 22 – Pockets for accessing buckle 2; 23 – Pockets for accessing buckle 3; 24 – Pockets for accessing buckles 6,7,8,9; 25 – Central zipper; 26 – Stirrup; 27 – Footstep; 28 – Shoes; 29 – Cuffs; 30 – Cuffs; 31 – Hooks for securing the suit to the boots; 32 – Pocket

Figure 6. Schematic of the Pingvin-3 suit (Barer 2008). Image credit to Barr, 2008 and Michael Barret (NASA) for communication facilitation.

Development of the SkinSuit

Building on the concept of loading suits as a low cost, volume and weight (<1kg) countermeasure against the loss of 1G axial loading in space, the Gravity Loading Countermeasure SkinSuit (GLCS) was conceived by James Waldie and Dava Newman at Massachusetts Institute of Technology (Waldie and Newman, 2011). It is comprised of a rigid yoke (chest) section over the shoulder designed to distribute the loading across the shoulders, with a bi-directional, porous weave running from this yoke (chest line) towards the feet where stirrups go under the feet to 'close the loop'. This design aimed to more closely replicate the magnitude and cumulative nature of gravitational loading experienced on Earth (Waldie, 2005; Waldie and Newman, 2011). Gz loading is progressively produced by increasing tension in the Gz axis fibres (with circumferential tension sufficient only to prevent suit slippage, estimated from material studies to be 10mmHg) using each circumferential fibre of its' elastic weave as a "belt" to produce hundreds of vertical stages; from the shoulders to the feet. Stirrups wrapped around shoes (or insoles) distribute the load across the sole, closing the 'elastic loop'. This provides a passive axial loading to the body, which may through cumulative wear support the cyclic disc compression/elongation on the spine, which is diminished in space.

The European Space Agency's SkinSuit has gone through several iterations to its current incarnation, the Mk VI SkinSuit, which is the present design chosen for spaceflight (Figure 7). During this time, it has been evaluated under several conditions. Several prototypes (Mk I/II) were studied for material properties during parabolic flight, another analogue for spaceflight which provides short (~22s) repeated bouts of microgravity (Waldie and Newman, 2011).

Figure 7. Evolution of the European Space Agency's SkinSuit to current Mk VI flight model. Image credit – European Space Agency and King's College London.

Loading and compatibility with both aerobic and resistance exercise was first evaluated in the Mk III GLCS. The axial loading imparted by the SkinSuit was assessed via pressure sensing insoles placed under the foot straps and shoulders in an upright posture, with an average Gz of 80% bodyweight recorded at the foot and 15% at the shoulder (Carvil *et al.*, 2013). Several studies of the Mk III GLCS went on to assess the compatibility with ISS exercise protocols, with both aerobic cycling (Attias *et al.*, 2017), running (Carvil et al. 2016) and resistance exercise (Carvil *et al.*, 2017) with approximately 1h wear time each. It was found to be compatible with all exercise modalities, with no thermal regulatory issues as reported with the Pingvin Suit, though it appeared to augment the oxygen cost of exercise and inhibit the range of motion at the shoulder. A further study on the Mk III GLCS effects on the haemodynamic responses to head down tilt reported that the compression did not impede the normal responses of cardiovascular compensation to an orthostatic stress (5 minute 6^0 HDT), though it increased subjective discomfort and notable shoulder compression was reported (Carvil *et al.*, 2014). That study also reported the first indication that acute wear of the suit reduced stature compared to normal clothes in the HDT analogue that has previously been used to induce elongation (Hutchinson *et al.*, 1995). This finding combined with the previous studies led to the further development of the GLCS into the SkinSuit to ascertain if it could be modified to provide a passive, tolerable axial load for a greater duration, which could then be assessed to determine its utility as an operational countermeasure for spinal elongation.

The SkinSuit has been modified from the GLCS to allow for rapid (~20s) donning and doffing for operational function and has been tested on the ground and during parabolic flight (Green *et al.*, 2014). This task can be completed without the need for additional assistance from another participant, which is an important requirement for spaceflight as astronaut time is limited. The material has been modified to improve durability and loading consistency (Kendrick and Newman, 2014). With this change in the material properties has resulted in an improved comfort and tolerability but subsequent decrease in the Gz load from ~0.8Gz to ~0.2Gz (Green *et al.*, 2015). Further padding has also been embedded into the shoulder along with a reduction in yoke size, an added zipped to allow male micturition, move of main zip from the front to the back and a simplification of the ankle stirrups to allow easier application of loading (Figure 7). These amendments contributed to what the Mk VI SkinSuit is today and its current requirement for further assessment to determine its applicability to provide comfortable and effective support to the body to mitigate spinal elongation.

Section 2.07 Summary

Human anatomy, in particular the spine has developed to facilitate upright posture and locomotion in a 1G environment, though structures are adaptable to the microgravity environment in space, long term this can result in deleterious effects to the body, including increased risk of disc herniation on return to Earth. To reintroduce axial loading in space novel low cost and volume countermeasures are required, with suitable analogues to evaluate them. The European Space Agency's

Mk VI SkinSuit is a lightweight loading suit comprised of a bi-directional elastic weave imparting a low-level passive 0.2Gz axial load at the foot. It is proposed as a spaceflight countermeasure to support the spine and its associated structures. However, no data exists on how it effects the spine in situations where it is unloaded and then reloaded with this technology. Thus, information is required to inform both the operational utility of the SkinSuit, but also the impact of load/unloading upon the spine with an appropriate spaceflight analogue.

Section 2.08 PhD aims

Based on review of the literature the overall aim of this PhD was to evaluate the impact of a novel axial loading countermeasure, the Mk VI SkinSuit upon stature, spinal structure and functionality utilising a suitable and accessible microgravity analogue. Specific aims were: -

1) Investigate the potential of a novel microgravity analogue platform, hyper-buoyancy flotation (HBF) to study unloading induced stature elongation
2) Explore the effects of wearing the SkinSuit with HBF to determine its efficacy as a countermeasure for elongation induced by unloading
3) Evaluate how the SkinSuits axial loading effects the spine with HBF
4) Understand how reloading the spine with the SkinSuit impacts both the geometry and kinematics of the lumbar spine

Five experimental Chapters are to follow. Chapter 3 addresses the first experimental aim of evaluating HBF, whilst Chapter 4 addresses both the first and second aims by introducing the SkinSuit. Chapter 5 addresses aim three using MRI, whilst Chapter 6 and 7 address the aspect of reloading the spine with the SkinSuit using ultrasound in the first pilot study followed by MRI and quantitative fluoroscopy. This is portrayed in the following diagram to help orientate the reader.

Chapter 3 - Study 1

- **Thesis aim addressed**: 1- Investigate microgravity analogue platform, hyper-buoyancy flotation (HBF)
- **Study title**: Evaluation of hyper-buoyancy flotation (HBF) as a microgravity 'unloading' analogue to induce stature elongation using stadiometry

Chapter 4 - Study 2

- **Thesis aim addressed**: 2- Explore the effects of wearing the SkinSuit with HBF to determine its efficacy as a countermeasure for elongation induced by unloading
- **Study title**: Investigating the effect of the Mk VI SkinSuits axial loading upon 8-hour hyper-buoyancy flotation induced elongation

Chapter 5 - Study 3

- **Thesis aim addressed**: 3- Evaluate how the SkinSuits axial loading effects the spine with HBF
- **Study title**: Examining the effects of wearing the Mk VI SkinSuit upon the spinal column using MRI after 8-hour hyper-buoyancy flotation

Chapter 6 - Study 4

- **Thesis aim addressed**: 3/4- Evaluate how the SkinSuits axial **re**loading effects the spine with HBF
- **Study title**: The effect of 4-hour SkinSuit induced partial axial reloading upon stature elongation and anterior intervertebral disc height as assessed by ultrasound after 8-hour hyper-buoyancy flotation

Chapter 7 - Study 5

- **Thesis aim addressed**: 4- Understand how reloading the spine with the SkinSuit impacts both the geometry and kinematics of the lumbar spine
- **Study title**: Exploring the effects of 4-hour partial axial reloading via the Mk VI SkinSuit upon the lumbar geometry and kinematics after 8-hour hyper-buoyancy flotation

Chapter 3. Evaluation of hyper-buoyancy flotation (HBF) as a microgravity 'unloading' analogue to induce stature elongation using stadiometry

Section 3.01 Introduction

During sleep the human body elongates resulting in a greater stature at the start of the day, which gradually attenuates due to loading (both movement and gravity) (De Puky, 1935). A study into these diurnal changes found in eight healthy men an average of 1.1% variation in stature attributed to circadian influences occurs ranging between 1.3-2cm (Tyrrell, Reilly and Troup, 1985). Seventy percent of the stature elongation happens within the first four hours of a eight hour sleep cycle, whilst upon waking (and resuming daily activity), 54% of this elongation is lost (after 3 hours and 45 minutes). This is attributed to changes in IVD height, spinal curvature and fluid redistribution i.e. compression of the heel pads (Foreman and Linge, 1989; Wing *et al.*, 1992). In space, this cycle of load/unloading is lost, resulting in prolonged unloading of the spine and stature elongation of up to 6.9cm being reported (Sayson *et al.*, 2013). This elongation has been associated with back pain, increased risk of musculoskeletal injury and elevated risk of disc herniation post flight (Johnston *et al.*, 2010).

Stadiometry has been used to determine stature elongation in space, however it can be prone to human measurement error, as historically astronauts marked the head and foot position on the wall and measured the distance between markers (Thornton, Hoffler and Rummel, 1977). More sophisticated systems to measure standing or seated stature attempt to control influencing factors including posture and gaze by utilising a solid measurement frame with adjustable poles to follow the participant's spine (Rodacki *et al.*, 2001). For supine height assessment, the gold standard for measuring supine height according to the Guinness book of records is "a stadiometer with a medical professional interpreting the results" (private communique with the Guinness book of records management team, 2015). Camera systems offer an alternative for assessment of stature in multiple postures and are currently being trialled in space by NASA (Sudhakar *et al.*, 2015), though these fixed systems (allocentric) are not easily portable nor are commonly found in clinical settings. As such several of the standardisation procedures i.e. gaze stabilisation, breathing cycle can be factored into stadiometry measurements using commercially available stadiometers, which can be brought to the subject to take standing and supine stature measurements (egocentric).

Analogues are currently utilised to study the effects of unloading induced by microgravity upon the body (Belavý et al. 2010). However current analogues may prove unsuitable for the evaluation of spinal countermeasures. Head-down tilt (HDT), the most commonly used analogue for spaceflight (Pavy-Le Traon *et al.*, 2007) induced stature elongation of 1.2cm after 24h that increased to 2cm after 3 days (Styf *et al.*, 1997). This is no more than that reported with 8h sleep (Tyrrell, Reilly and Troup, 1985). Recent HDT studies have also suggested that this vector does not best represent the haemodynamic situation in microgravity, as the blood pressure gradients are closer aligned to a supine horizontal state, not a weight-bearing head-down position (Hargens and Vico, 2016). Whilst HDT participants are easily accessible and transportable on/off the inclined bed, an environment where a Gz vector is not imposed is desirable.

Dry immersion uses a lined barrier between the participant and the water to prevent skin maceration associated with wet immersion (Barr, Clement and Norsk, 2015). Participants are placed on the surface of this 'barrier', before being lowered into the water via inbuilt hydraulic lifts. Similar to wet immersion the participant's head remains out of the water but the body sinks and becomes flexed at the hips. Three-days of dry immersion has been found to induce significant lumbar IVD swelling and accompanying lower back pain (assessed via a 0-10 visual analogue scale) in 92% of participants, (Treffel *et al.*, 2017). Similar reports of combined elongation and accompanying back pain were reported a three day HDT study (Styf *et al.*, 2001). Participant's accessibility is however limited with dry immersion, making any spinal or stature measurements whilst immersed impractical, thus stature elongation during dry immersion has never been documented. Also dry immersion has been reported to be uncomfortable for the participant, inducing a compression on the chest from the weight of the water (Dornelas et al. 2014), disorientation and motion sickness (Barr, Clement and Norsk, 2015). Therefore, recording of participant comfort and subjective back pain is an important factor to consider in analogue evaluation.

Restrictive environmental stimulation therapy flotation (REST), is not an analogue platform but a type of therapy. It utilises a hypersaline solution of water and Epsom salts to increase the density of the water to induce a buoyant state, though the subject cannot stay in the tank long due to the maceration of the skin and risk for drowning (Hill *et al.*, 1999). Data on its effects on the spine are confined to subjective accounts of reduced back pain (Kjellgren *et al.*, 2001).

Recently, a novel system, termed hyper-buoyancy floatation (HBF) was devised at King's College London combining principles from REST and dry immersion. A waterbed is partially filled with hypersaline water; a mixture of Magnesium sulphate and water to a $1.7gcm^3$ density maintained at a thermo-neutral 34^oC temperature using an electric heater (Kjellgren *et al.*, 2001). It is surrounded by a 2x1.2m MDF frame for containment separated by a plastic sheet for safety precautions in case of leaks. A stadiometer is connected to the MDF frame to take supine, allocentric stature measurements. Unlike REST and wet immersion where participant stay is limited to a few hours due to skin maceration (Barr, Clement and Norsk, 2015), the barrier from the waterbed provides a dry, buoyant flotation that can be used for long durations. This keeps the participant in a supportive, near horizontal flotation where the body mass sinks proportionally into the bed. A cotton sheet (1mm) is also placed on top to protect the bed from puncture and an optional blanket provided for thermal comfort (Figure 8).

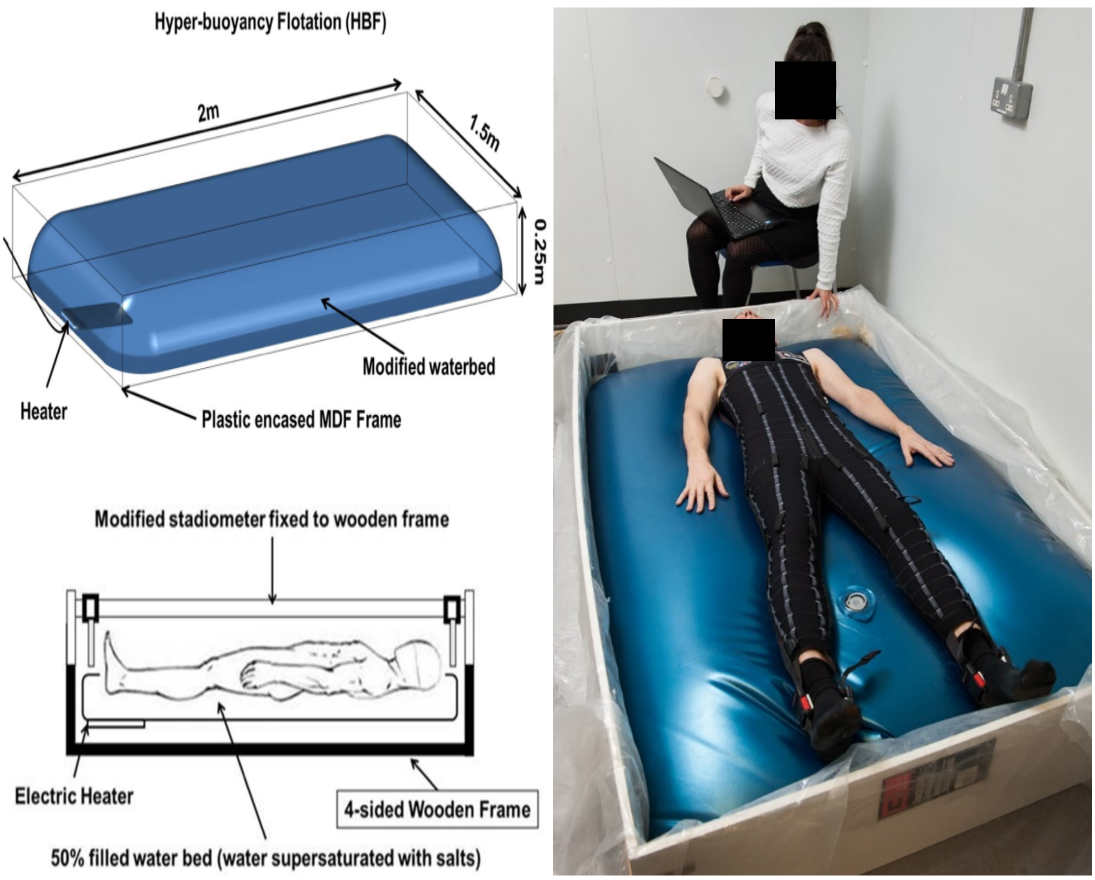


Figure 8. Schematic of the HBF (top left), participant stature assessment during HBF (bottom left) and participant position on the HBF (right). Image credit King's College London.

However, no data exist on HBF's effectiveness in inducing significant elongation. As such the study of stature change from HBF, using time periods previously evaluated during sleep studies, 4h and 8h respectively (Tyrrell et al. 1985) requires investigation to assess potential suitability as a spaceflight analogue. Therefore, the hypotheses are that both 4h and 8h HBF will induce a stature elongation greater than or equal to that reported in 24h head down tilt and 8h sleep.

The aim of this pilot study was to:

1) Evaluate the effects of both 4h and 8h HBF upon participants' stature using stadiometry and comfort with visual analogue scales.

Section 3.02 Method

Experimental Approach

Ethical approval was sought and approved by the King's College London ethics committee (BDM/13/14-107). Based on the mean and standard deviations reported from a previous analogue study on stature elongation pre-vs. post (Styf *et al.*, 1997), power calculations (β=0.8, α=0.05) indicated a sample size of 6 participants was required to determine an elongation effect (0.97). Sample size calculations were run using G*Power (Heinrich Heine, University of Düsseldorf, Germany) (Faul *et al.*, 2007). Two studies were planned, one of 4h duration and one of 8h, with the durations chosen based on the stature elongation recorded from a previous sleep study (Tyrrell, Reilly and Troup, 1985). Due to the 8h time commitment of lying on the HBF only males were recruited, where a non-invasive system facilitating micturition, whilst supine, could be implemented. The main outcome measure was stature elongation, for which intra-observer repeatability was performed.

Repeatability of stature measurement

Ten healthy volunteers were asked to have their standing height measured five times, by the same observer, both from a standard standing position and after having laid supine for five minutes using a commercially available stadiometer (Cambridge measuring systems, UK). This was to factor in unloading of the body, with measurements taken within 15s of transition from supine to attenuate influence from heel pad deformation (Foreman and Linge, 1989). Head positon was stabilised each time with participants asked to fix their gaze on the horizon (Rodacki *et al.*, 2001). All standing stature measurements were taken at tidal inspiration and expiration by the same observer. Intra-class correlation coefficients (ICC) were calculated using the variance between two repeated measures of 20 heights, plus the residuals from a two-way mixed ANOVA. The alpha was set at 0.05, with 95% confidence intervals (CI). Standard error of measurement (SEM) was calculated by taking the standard deviation (SD) * $\sqrt{(1\text{-ICC})}$, with the range taken as the most extreme deviation between repeated measurements. Minimal detectable change was calculated by the formula $1.96*\sqrt{2}*\text{SEM}$ (Table 1), whilst this was similar between breathing in and breathing out, the accuracy (based on the range between repeated measures) was considerably smaller with inspiration, therefore inspiration measurements will be reported going forward.

Table 1. Intra-observer reliability, variation of measurement and minimal detectable change of stature measurement by the author.

Parameter	ICC (95% CI)	Mean (cm)	SD (cm)	SEM (mm)	Range (cm)	MDC (mm)
Stature Breathing In (mm)	1 (1)	172.1	0.7	0.02	0.3	0.06
Stature Breathing Out (mm)	1 (0.99-1)	172.1	0.9	0.03	0.6	0.09

Participants

Fourteen subjects were recruited for both the 4h (♂=10, 27±5yrs, 1.76±0.07m, 75.4±8.5kg; ♂=4, 30±11yrs, 1.64±0.06m, 58.9±1.79kg) and 8h HBF trials. (♂=14; 35±2y; 1.79±0.08m; 81.2±7.9kg). Each gave written informed consent to participate in the study and had no history of neurological, cardiorespiratory and/or psychological disorders. None of the participants were in pain, or knew/suspected that they were pregnant (4h study only) and were asked if they had a history of severe, chronic back pain, discectomy or had recently sought treatment for musculoskeletal issues. If so, they were excluded. Participants were instructed to abstain from vigorous exercise and alcohol for at least 24 hours before the study and caffeine for at least two hours prior to each session.

Protocol

Two separate testing sessions, identical apart from duration, one being 4h the other 8h, were performed in a temperature controlled laboratory (23.9 ± 0.2^{o}C) with the HBF maintained at a thermo-neutral temperature (34–35° C) (Kjellgren *et al.*, 2001). Participants were instructed to wear comfortable, non-skin-tight clothing for testing and could view films projected on an overhead screen to minimise neck movement and straining. Upon arrival participants filled out a back pain questionnaire (ISS pre-flight questionnaire, Appendix- Section 10.01) before being familiarised with the study protocol.

Measures of standing stature were taken and compared pre-vs. post using the stature recorded at maximum inhalation with a commercially available stadiometer (Cambridge measurements systems, UK). Supine stature was recorded every 30 minutes whilst on the HBF (Figure 8) using a custom built allocentric stature measurement system. Participants were asked to rate their thermal comfort (Gagge, Nishi and Nevins, 1976), movement discomfort (Corlett and Bishop, 1976), body control (Cooper and Harper, 1969) and to rate/mark on a body pain map any localised pain and its intensity, pre, post and every 30 minutes during HBF (Figure 9).

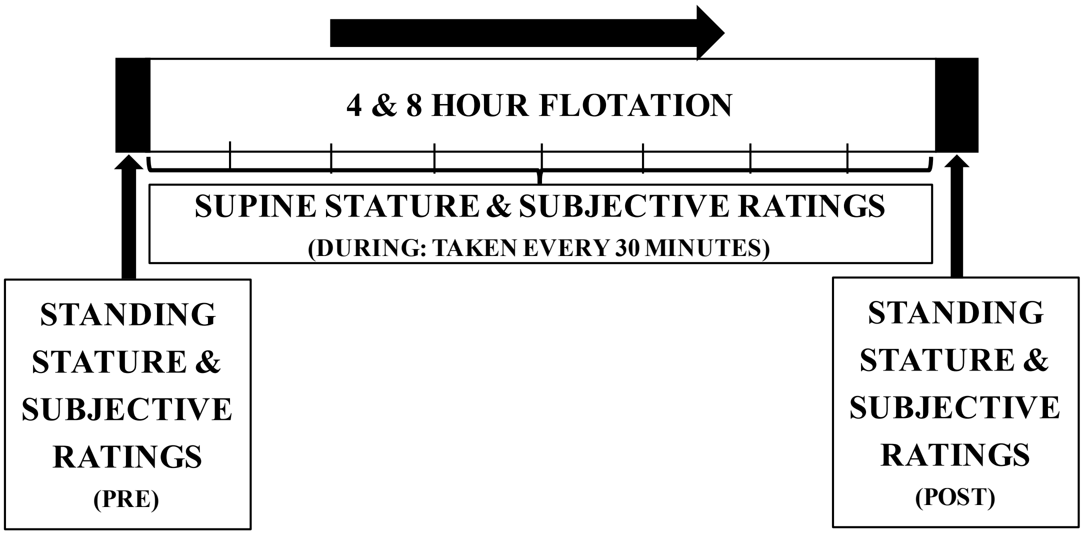


Figure 9. Schematic diagram of study protocol detailing when stature measurements and subjective rating scales were taken.

Following the experiment seven participants from each of the 4h and 8h HBF groups volunteered to record their stature pre-and-post 8h sleep. They measured their height three times upon waking after 8h sleep (with assistance from someone at home) using the same stadiometer (Cambridge measurements systems, UK). The average of these measured was used to report the elongation resulting from sleep.

Data analysis

Choice of statistical test was determined by the type of data (subjective vs. objective) and having assessed normality by visually inspecting histograms and whether the skewness and kurtosis ratio lay below or above 1.96/-1.96 (Fallowfield, Hale and Wilkinson, 2005). Data is expressed as either means ± SD (stature) or median ± interquartile range (subjective ratings). Pre vs. post stature was compared using a paired t-test, whilst subjective questionnaires were compared with a Wilcoxon test. An independent samples t-test was run to compare the elongation from 4h and 8h HBF. Fourteen participants (seven from each of the 4h and 8h groups) measured their height following sleep at home, this change was compared with their respective elongations from the HBF using a paired t-test. Measurements of stature and subjective scales during HBF were assessed using a one-way repeated measures ANOVA or Friedman's respectively. Self-reported pain ratings were provided over two regions of the back and were classified as the neck (C1/head-C7/collar bone) and lower back (T12-S1). Statistics were performed using Statistical Package for Social Sciences 24.0 (SPSS IBM, Chicago, IL, USA) with significance assumed when $p < 0.05$.

Section 3.03 Results

All participants safely completed their respective times on the HBF sessions. Significant stature elongation was recorded (pre-vs. post) after both 4h and 8h HBF and in the sleep study (Table 2). Between 4h and 8h HBF there was a trend for greater elongation with 8h (p=0.110). The participants who measured their height following 8h sleep at home were observed to have greater elongation with both 4h (1.6±0.5 vs. 1.3±0.6cm; p=0.15) and 8h (2.3±0.7 vs. 1.7±0.4cm; p=0.11) HBF compared with their respective sleep measurements.

Table 2. Standing height (mean±SD) recorded PRE-vs. POST HBF.

Time	PRE (Inhalation; cm)	POST (Inhalation; cm)	Δ (Inhalation; cm)
4h HBF (n=14)	174.5 ± 8.8	176.2 ± 9.2*	1.7 ± 0.8
8h HBF (n=14)	178.8 ± 7.5	181.0 ± 7.8*	2.2 ± 0.6
Sleep (n=14 $^{\$}$)	178.8 ± 6.3	179.3 ± 6.1*	1.4 ± 0.6

*Significant (p<0.001) increase PRE-vs. POST. $^{\$}$ seven participants from the 4h and 8h HBF trial.

There was a significant increase in participant elongation from the start of both 4h [$F_{(7,91)}$= 5.1, p<0.001] and 8h HBF [$F_{(15,165)}$= 4.8, p<0.001] of 1.7±1.1cm and 1.3 ± 1.3cm respectively (Figure 10).

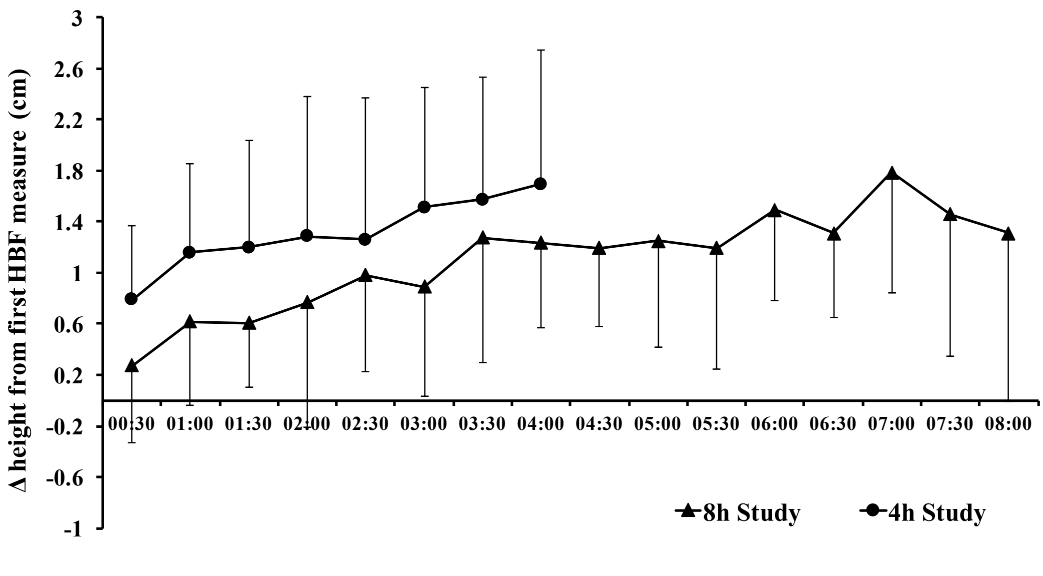


Figure 10. Delta height (mean±SD) from first HBF measure during the 4h and 8h HBF protocol.

Subjective ratings

There were no significant changes in the subjective rating of thermal comfort, movement discomfort or body control during or following 4h or 8h HBF. During HBF minor discomfort was noted in the neck in the 8h trial (χ^2=53.5; p<0.001), manifesting after 5h's of HBF in eight participants (1.25 [0-2.5]). Self-reported ratings of lower back pain, were significantly (z=-2.7; p=0.02) higher post 8h HBF (0[0] vs. 1.25 [0-2.5]). Furthermore, during HBF ratings of lower back pain significantly increased over time (Figure 11) in both the 4h (χ^2=20.1; p=0.01) and 8h (χ^2=112.8; p<0.001) trials.

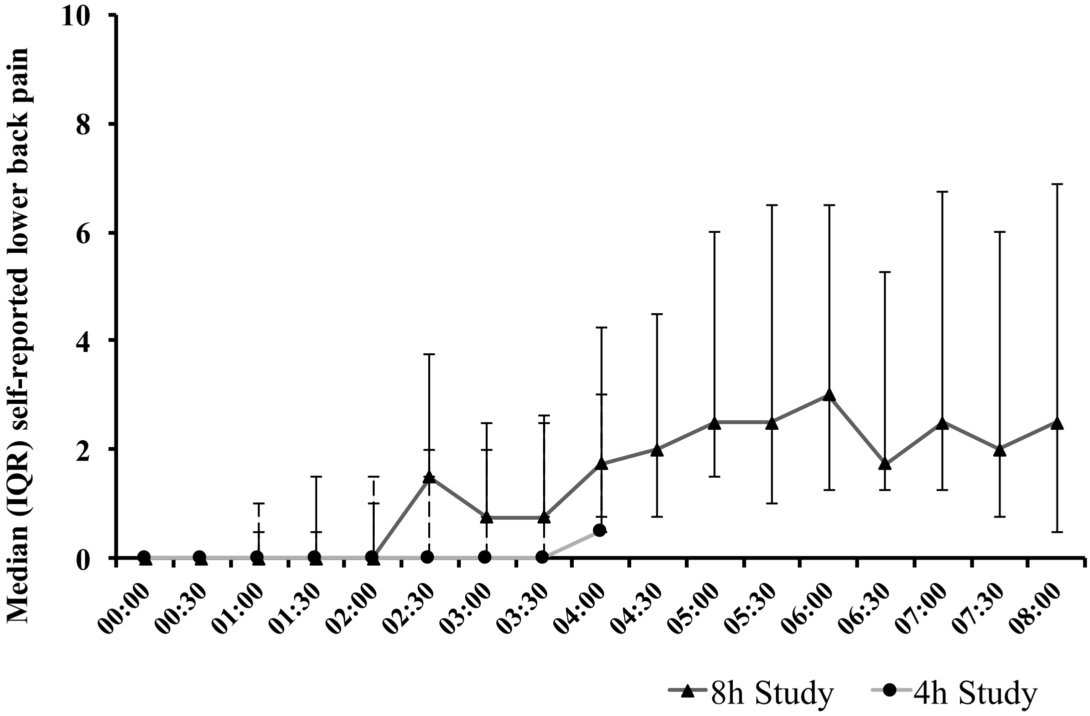


Figure 11. Rating (0-10) of lower back pain (median±interquartile range) recorded during the 4h and 8h HBF protocol.

Section 3.04 Discussion

The main findings of the present study were that HBF, both 4h and 8h sessions, resulted in a significant increase in stature elongation that was equal to and/or greater than that reported with 8h sleep (Tyrrell, Reilly and Troup, 1985) and 24h HDT (Styf *et al.*, 1997). A trend for a greater elongation was observed after 8h vs. 4h HBF. No tolerance issues arose from lying on the HBF with all participants completing their respective HBF sessions. Lower back pain was reported arising after 5h of HBF, however this resolved upon standing and movement.

Effects of HBF on stature

Studies measuring stature changes have shown after 24h of HDT an average of 1.2cm of stature elongation (Styf et al, 1997). Compared to HDT, HBF induced a greater amount of elongation given the comparatively reduced time of flotation (4h/8h HBF vs. 24h HDT). HDT (6^o) has been the standard method of facilitating both short and long duration microgravity analogue studies since its first use in the 1970's (Budylina, Khvatova and Volozhin, 1976), resulting in a significant contribution to the knowledge and development of spaceflight countermeasures (Pavy-Le Traon *et al.*, 2007). This discrepancy maybe due to the orientation of the participant in HDT effecting elongation, or a lack of rigour in the measurement of stature. A HDT study has shown lumbar musculature deconditioning and suspected IVD disc expansion, associated with spaceflight after 60 days of HDT (Belavý, Armbrecht and Felsenberg, 2012). However, hypertrophy of the cervical muscles and thoracic discs has also been reported with HDT (Belavý *et al.*, 2013), which is not associated with spaceflight, but is likely related to the head down position of the participant. Therefore, despite its wide utilisation HDT may not be the optimal analogue to facilitate unloading/loading evaluations of the spine (Hargens and Vico, 2016). However, HDT studies have been run successfully for far greater lengths of time (up to several months) to evaluate countermeasures, whereas the present study was limited to 8h. Therefore, HBF would require further investigation to determine the effects upon the spine both over 8h with imaging and over a longer time period to support its implementation as a human spaceflight analogue.

Participant's stature elongation following 8h sleep ranged between 1.3-1.7cm. However, this was not a controlled sleep assessment of diurnal fluctuations in stature as performed in the literature, but rather gives further context, in conjunction with the literature on sleep induced elongation relative to the participants who undertook HBF. During a controlled study on diurnal fluctuations of stature, there was an average of 1.3cm elongation after the first half of sleep (~4h) rising to 2cm after 8h, in eight studied participants (Tyrrell, Reilly and Troup, 1985). Compared with the diurnal study run by Tyrell's group, the present study did have higher subject numbers and also included female participants in the 4h trial, but stature measurement taken after participant's sleep were not subject to the same rigor i.e. a participant's friend took the measurements for practicality, as such poor inter-rater reliability is likely a contributing factor to error even though an average was taken from three measurements. The potential for measurement bias is also a factor as such results from this adjunct sleep assessment should be treated with caution, though they are within the ranges reported from literature. Both 4h and 8h HBF flotations did induce elongation on a par with or greater than that recorded after participants 8h sleep and that reported in literature. It is noted that whilst the minimal detectable change of 0.06mm with the present study's commercial stadiometer was able to detect a significant elongation with HBF, the standard deviation of 0.7mm is far higher than that reported with the custom-built measurement system which takes into account intra-subject variations in posture and curvature (SD: 0.18mm) (Tyrrell, Reilly and Troup, 1985; Reilly and Freeman, 2006). Therefore, where available these systems should be employed to improve the standardisation of stature measurement.

In contrast to dry immersion, HBF provides an accessible platform where measurements can easily be taken both during and off the bed. With dry immersion, as the subjects are immersed, no direct access is available to measure their height during immersion, as they are in a non-standardised flexed position in the water, similar to the foetal position in space. The lack of reported stature measurements pre-and post-dry immersion owe partly to complexities of getting the subject out of the tank for measurement without substantial effort/disruption. As such comparable measures with HBF are not available (Navasiolava *et al.*, 2011). However, a recent dry immersion study investigating pre vs. post changes in the spine found significant lumbar IVD swelling after 3 day immersion (Treffel *et al.*, 2016). In this present study, only stature measurements were taken to determine the effect of HBF on stature elongation.

Stature measurements have been associated with changes in the spinal length (Brinckmann *et al.*, 1992). One study that used a seated stadiometer to isolate the effect of posture on spinal height, in conjunction with MRI to characterise the spinal length changes (Kourtis *et al.*, 2004). Whilst stature measures have been used to infer changes in IVD height in previous research (Lewis and Fowler, 2009), the lack of imaging in the present study is a limitation, as such this is warranted in future investigations. Studies discussed in the following Chapters will integrate imaging modalities with stadiometer measurements to facilitate the evaluation of countermeasures.

Effects of HBF on subjective measurements

Reports of subjective discomfort during HBF were low, in contrast to those reported with dry immersion, where back pain, nasal congestion and head heaviness are reported in the first two days (Tomilovskaya, 2013). This might be due to the lack of compression on the thoracic cavity and head up position (Navasiolava *et al.*, 2011), in which the weight of the water on the chest can restrict lung function (Dornelas et al. 2014). Also, being immersed in a large temperature controlled body of water may impede the thermal comfort of the individual. This is important to consider with following studies where countermeasures are integrated for evaluation, if the analogue was not suitable for the subjects to lie on for an extended period, such evaluations would be hindered. Due to the low discomfort and multi-platform compatibility, HBF might also facilitate a short-term bed rest study (3-7days) to study longer periods of unloading analogous of space on the spinal structures, as previously performed in bed rest studies.

During both 4h and 8h HBF some participants reported minor lower back pain (Figure 11), which was more intense with 8h HBF. Back pain has been reported with both spaceflight (Wing *et al.*, 1991) and head down tilt (Hutchinson *et al.*, 1995). In spaceflight, 68% of surveyed crew members reported acute lower back pain (Wing *et al.*, 1991), which is thought to be attributed to disc expansion and resultant soft tissue stretching (Sayson and Hargens, 2008). In the study of stature elongation associated with HDT, back pain was also reported, increasing in intensity from day 1 to day 3 with stature elongation (that plateaued until the end of the HDT period; (Hutchinson *et al.*, 1995). The trend for increased elongation in 8h HBF suggests it might provide a more suitable model for evaluating elongation and axial loading countermeasures than 4h HBF. Whilst HBF induced mild, reversible back pain as self-reported by the participants, it is important to note that whilst the mechanism might be related to elongation, the understanding of the pathophysiology of back pain, due to its multifaceted nature (Flor, 2002) is out of the remit of both the present study and this thesis. However, in a 3-day dry immersion study increased disc swelling was accompanied by reports of back pain via a visual analogue scale (Treffel *et al.*, 2016), which is a potential mechanism in this study. A limitation of the present study was that only stature was recorded; future studies should seek to characterise the effect of HBF unloading on the IVD's to determine if there is an increase in IVD swelling, which could be a contributing factor in the development of back pain.

In the current study 8 individuals also reported minor (1.25 [0-2.5]) discomfort in their neck after 5h in the 8h HBF trial. The reason for this was not investigated however logically it might be attributed to similar purported factors as spaceflight induced back pain, that of soft tissue stretching (Sayson *et al.*, 2013a). In a study of optimal pillow heights, the lordosis of the neck increased with elevated pillow height affecting both the cranial alignment and cranial-cervical distribution of pressure (Ren *et al.*, 2016). Changes in these factors are thought to influence comfort and quality of sleep (Ren *et al.*, 2016), as such alterations in these parameters could be contributing factors to the self-reported neck pain in the present study. Further study into the effect of unloading and loading on cervical disc heights using imaging should be investigated, as the cervical region is also a risk area for herniation in astronauts (Johnston *et al.*, 2010), with relatively little known (Belavy *et al.*, 2016).

Conclusion

HBF provides a novel platform that provides levels of elongation with 8h, that are on par with or greater than that observed in 8h of diurnal sleep and in one day of

HDT. As such, the supine, buoyant position enabled by HBF may provide an alternative microgravity analogue, though imaging studies are required.

Further HBF analogue studies using the 8h protocol which induced a trend for greater elongation than 4h, are warranted to study stature elongation and spinal changes in conjunction utilising imaging modalities.

With a significant increase in stature induced through 8h HBF, the integration with axial loading countermeasures in combination with imaging assessment can be undertaken (Chapters 4 and 5). This will act to compare the effect of unloading and loading the spine, which would then better inform the utility and operational use of these experimental spaceflight axial-loading countermeasures which have been trialled in acute microgravity analogues i.e. parabolic flight (Figure 12).

Figure 12. Experimental testing of the Mk V SkinSuit during parabolic flight with ESA Astronaut Thomas Pesquet, performed to optimise design and characterise SkinSuit loading and comfort, prior to long duration (8h) ground model testing and spaceflight operational testing. Image Credit: ESA.

Chapter 4. Investigating the effect of the Mk VI SkinSuits axial loading upon 8-hour hyper-buoyancy flotation induced elongation

Section 4.01 Introduction

The loss of axial loading imparted by Earth's gravity results in substantial stature elongation up to 6 cm (Thornton, Hoffler and Rummel, 1977). This has been associated with reports of in-orbit back pain (Wing et al. 199) and difficulties with extravehicular (EVA) spacesuit donning (Nicogossian, 1989). Prolonged loss of loading has also been observed to increase markers of disc degeneration (Jin *et al.*, 2013; Sayson *et al.*, 2015), increase disc swelling with accompanying back pain (Treffel *et al.*, 2016) and led to atrophy of the paraspinal muscles (Chang *et al.*, 2016; Belavý, Gast and Felsenberg, 2017). These deleterious effects recorded after spaceflight are likely contributing factors to the reported 4-fold increase in the risk of disc herniation in the astronaut population on return to Earth (Johnston *et al.*, 2010). In order to support long duration human spaceflight exploration, low-cost, low-volume countermeasures require evaluation, using an appropriate analogue platform, to counter or attenuate the deleterious effects of spaceflight associated with the spine.

Hyper-buoyancy flotation (HBF) holds promise as a potential platform to evaluate spinal countermeasures (Chapter 3). It has resulted in significant stature elongation, in excess of that that reported with 24h head down tilt whilst not imposing a Gz vector cranially (Styf *et al.*, 1997) and still providing accessibility too and for participants. Stature elongation in microgravity (discussed further in Chapter 3) has been suggested to be principally due to elongation of the spine (Wing *et al.*, 1992). In space, spinal elongation is thought to be induced by intervertebral disc swelling (IVD) and/or flattening of the spinal curvatures though only measurements of stature elongation have been performed in space (Sayson *et al.*, 2013). Studies on Earth investigating stature elongation with atereophotography, have found that up to 40%

can be attributed to the lumbar spine (Wing *et al.*, 1992). In an overnight study measuring the distance between the lumbar processes (L1-L4) using ultrasound after 8h sleep, an increase of 5.3mm was observed (Ledsome et al. 1996).

However, whilst stadiometry can be used to infer changes in spinal length, it cannot identify whether elongations occurs due to changes happening with IVD swelling or spinal curvature loss, therefore imaging is required (Lewis and Fowler, 2009). Ultrasound has been piloted in spaceflight studies to image the anterior height of the cervical and lumbar discs but does not provide information on the whole disc. (Marshburn et al, 2013). Magnetic Resonance Imaging (MRI) is the gold-standard imaging modality for assessment of the spine including IVD geometry, it does not use ionising radiation and is able to differentiate soft tissues, therefore visualising the IVDs (Wassenaar *et al.*, 2012). However even small amounts of ferrous metals in clothing (i.e. zips) can distort MRI images and present a safety hazard to the patient and scanner. Therefore, in the evaluation of spaceflight countermeasures containing ferrous elements alterative imaging is needed.

The geometry of spinal structures can be assessed with dual-energy X-ray absorptiometry (DEXA) which provides a relatively low radiation dose (compared to CT scanning) and allows for exploratory imaging in conditions where MRI is not possible due to metallic contaminants. This technique is normally used for assessing bone density and body composition (Mazess *et al.*, 1990), though it can be adapted to study vertebral morphometry to perform an intervertebral analysis (IVA) (El Maghraoui and Roux, 2008). By visualising the vertebral bodies in a sagittal plane, it allows the measurements of the distance between the lumbar vertebrae; the intervertebral space, in-vivo. This technique can be used whilst wearing clothing which contains ferrous metals, such as those in components of the European Space Agency's SkinSuit such as zips and buckles.

The Mk VI SkinSuit is an evolution of the GLCS detailed in the literature review (Chapter 2, Section 2.06). It has been proposed as a possible countermeasure against spinal elongation in space through the re-introduction of axial loading. It imparts axial loading via a bi-directional elastic weave (Elastot 200) which has a high material tension in the vertical axis providing elastic loading, shoulder to foot and a low circumferential tension to stage the loading and prevent suit slippage (Waldie and Newman, 2011). Each SkinSuit is tailor-made based on measurements of participants vertical and circumferential anthropometrics, with arrestor ribbons sown at 4cm intervals to prevent overstretch of any one segment. Buckles have built in catches for desired corresponding loading which is measured at the foot using pressure sensors (*XSENS ForceShoe*™), which have also been used in space to assess loading during ARED exercise (NASA, 2017) and in parabolic flight (Green *et al.*, 2014) to assess loading. The SkinSuit can be doffed to halfway within 10s via a long cord connected to the back zip which has been evaluated in parabolic flight (Green *et al.*, 2014) (Figure 13).

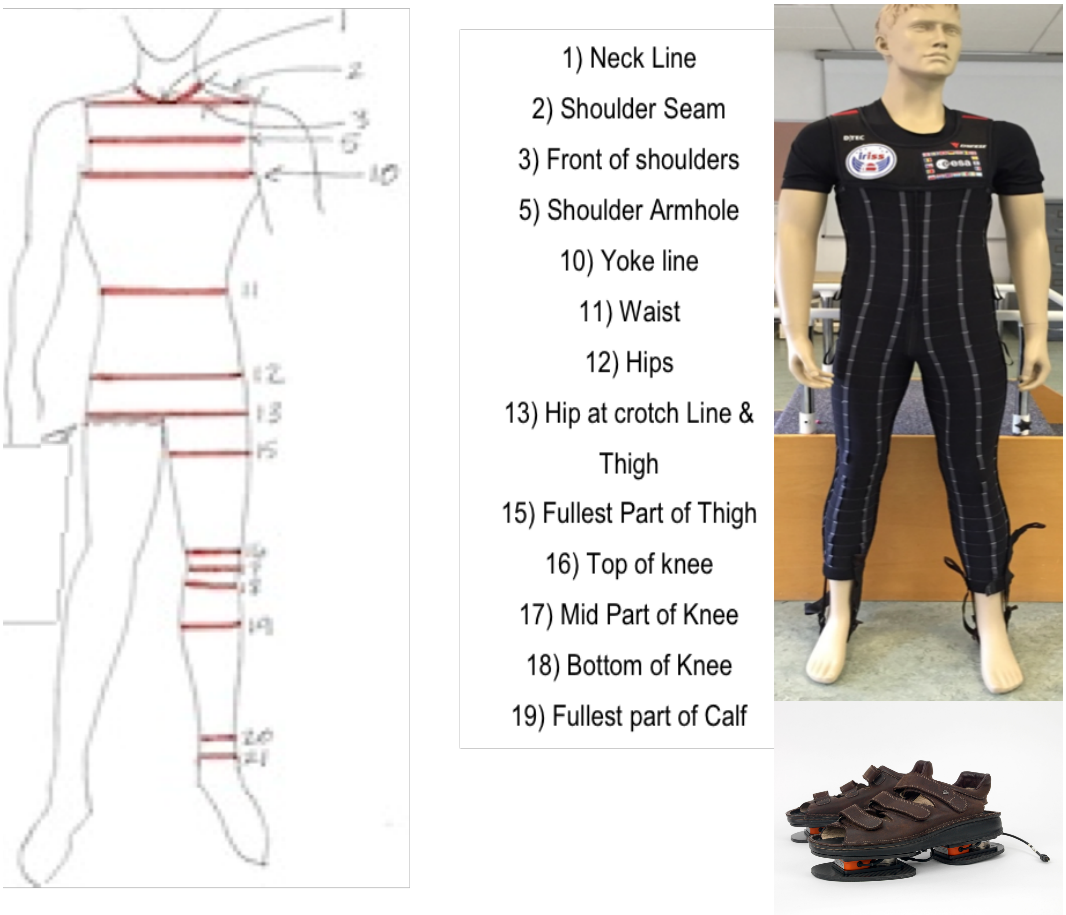


Figure 13. Original drawing from tailoring designs (left – image credit CostumeWorks, Boston, MA, USA) and the current Mk VI SkinSuit (right – image credit ESA and King's College London) with Forceshoes (bottom right – image credit XSENS/NASA).

A previous version of the SkinSuit, the Mk III, imparted higher loading ~0.8Gz but could only be worn for short periods of time due to high discomfort (Carvil *et al.*, 2017). The Mk VI SkinSuit therefore was optimised for operational use in space by decreasing the imparted loading to 0.2Gz at the foot and amending the suit's ergonomics to improve tolerability for long-term wear (Green *et al.*, 2015).

However, no study has determined the SkinSuit's efficacy in attenuating elongation using an appropriate microgravity analogue, nor assessed the tolerability of wearing this version for long periods of time associated with significant elongation.

Therefore, the present study investigated the effect of the Mk VI SkinSuit on stature elongation and lumbar IVD height during an 8-hour period of HBF, compared to no SkinSuit (control). The hypothesis is that the axial loading imparted by the MK VI SkinSuit will partially attenuate the effects of unloading on stature elongation induced by 8h HBF.

The aims of this pilot study were to: -

1) Investigate whether stature elongation induced by 8h HBF is attenuated through SkinSuit wear via stadiometry taken pre-vs. post and report overall subjective comfort of wear with visual analogue scales
2) Assess the applicability of using exploratory DEXA imaging to investigate whether the SkinSuits' axial loading reduces the height of the lumbar IVDs

Section 4.02 Methods

Experimental Approach

Ethical approval was sought and approved by the King's College London ethics committee (BDM/13/14-107). The study consisted of two testing sessions in a randomised crossover design. Nine Mk VI SkinSuits were able to be manufactured for the present study for male volunteers only, due to the 8h time commitment in a static supine position. The main outcome measures were stature and IVD height, for stature the same stadiometer and method from Chapter 3 was used with a minimal detectable change (MDC) of 0.09mm, for IVD height the repeatability from 12 separate L1-L3 images was calculated using the same protocol listed in Chapter 3 (Table 3).

Table 3. Intra-observer reliability, variation of measurement and minimal detectable change of IVD disc height (mm) by the author.

IVD height	ICC (95% CI)	Mean (mm)	SD (mm)	SEM (mm)	Range (mm)	MDC (mm)
Anterior (mm)	0.906 (0.824-0.951)	10.3	0.68	0.21	3.4	0.58
Middle (mm)	0.912 (0.832-0.954)	9.5	0.38	0.11	1.2	0.32
Posterior (mm)	0.794 (0.632-0.889)	6.9	0.25	0.11	1.0	0.3

Participants

Nine healthy male subjects gave written, informed consent to participate in two 8h HBF sessions acting as their own controls (30±5y; 1.77±0.07m; 74.9±8.1kg). None reported a history of neurological, cardiorespiratory and/or psychological disorders, nor severe, chronic back pain, a discectomy or had recently sought treatment for musculoskeletal issues. Prior to the experiment each came for a familiarisation session, where they were measured for a Mk VI SkinSuit by taking circumferential measures every 2cm, ankle to chest and several additional anthropometric measurements including the chest/yoke line detailed in Figure 13. SkinSuits were fabricated (Dainese, Italy) and donned by the participant prior to testing to ensure appropriate fit. They were asked to abstain from alcohol, caffeine and vigorous exercise in the day leading up to the study.

Protocol

Participants were requested to attend the laboratory twice during the day (8am-4pm) and lay on the HBF for 8h, followed by supine transit via patient trolley to the Osteoporosis unit at Guy's Hospital, London. They wore normal gym clothes in one session (non-SkinSuit- control) and the Mk VI SkinSuit with stirrups tightened to impart axial loading in the other (SkinSuit). The axial loading for each SkinSuit was assessed with stirrups wrapped around a pressure sensing shoe (ForceShoes, Xsens, Netherlands) and pulled to the designed vertical stretch of the SkinSuit for that individual. The average loading from the Mk VI SkinSuit whilst supine was on average 0.13±0.03 (range: 0.09–0.18) Gz.

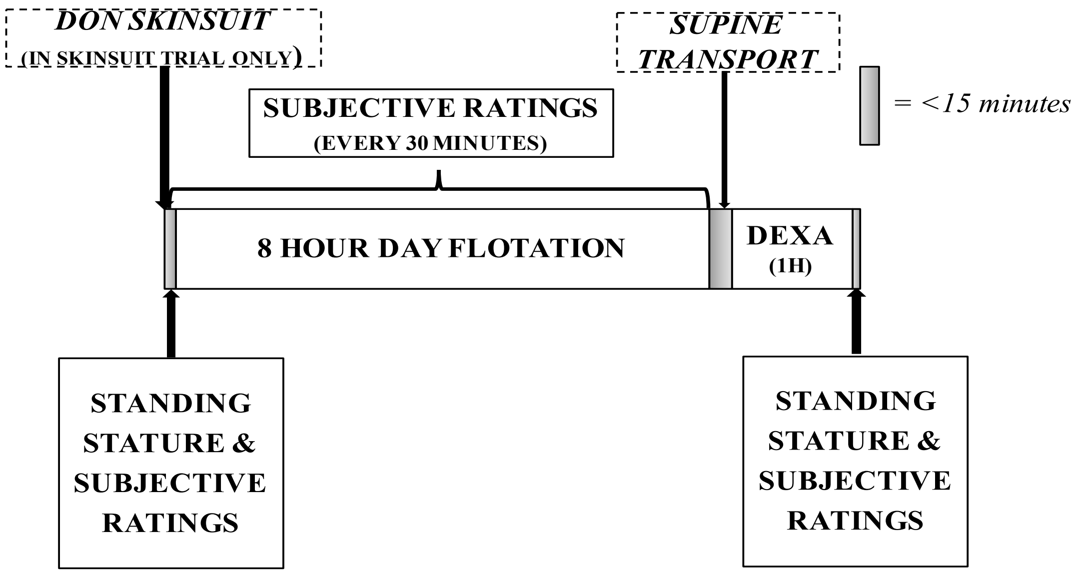


Figure 14. Schematic diagram of study protocol detailing when stature measurements and subjective rating scales were taken, followed by transport to DEXA scanning.

Subjective thermal comfort (Gagge, Nishi and Nevins, 1976) movement discomfort (Corlett and Bishop, 1976), body control (Cooper and Harper, 1969) and back pain ratings (Appendix) were requested every 30 minutes whilst on the HBF, before (PRE) and after (POST). Stature was recorded before (PRE) and after (POST) 8h HBF using stadiometry (Cambridge measurements systems, UK). Immediately after HBF participants were transferred off the HBF whilst maintaining a supine position using a stretcher and via a patient transfer trolley, transported to the DEXA scanner (Hologic Discovery QDR 4500) located in Guy's Hospital. Bone densitometry of the lumbar region facilitated intervertebral assessment (IVA) of the height between the vertebral bodies via a sagittal scan (L1-L4) (Figure 15). Prior to scientific analysis images were reviewed by a radiographer in order to report any underlying spinal pathology.

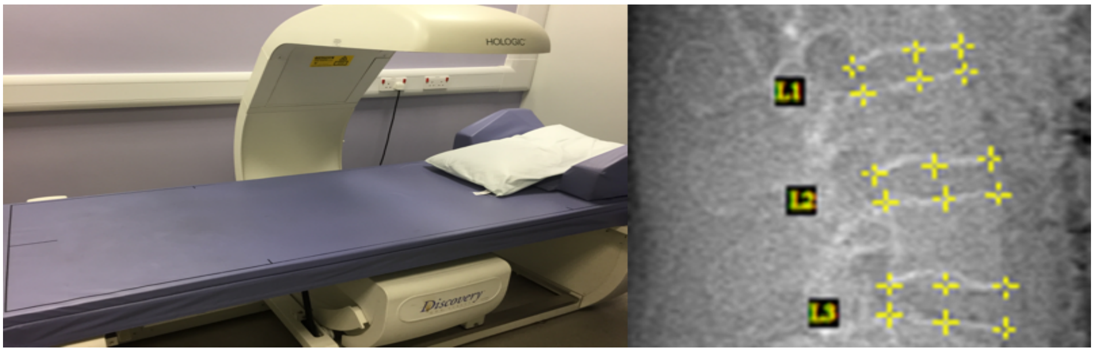


Figure 15. Setup for a sagittal scan of the lumbar spine using a DEXA scanner (left) and the mark-up for calculating IVD heights on the vertebral corners (right). Image Credit – Guys and St Thomas NHS Foundation Trust.

Data Analysis

All data was anonymised by random QR code generation with the researcher blinded to images prior to analysis. Data were assessed for normality by a visual check of histograms and by assessing whether the skewness and kurtosis ratio lay below or above 1.96/-1.96 (Fallowfield, Hale and Wilkinson, 2005). Anterior, middle and posterior markers were placed on the caudal and celaphid sides of each vertebrae (Figure 15; APEX DICOM, Hologic Discovery, Massachusetts, USA) to calculate the heights of IVD spaces between L1/L2, L2/L3 and L3/L4. Only L1-L4 vertebrae were visible in all participants, not L5, thus only these three disc spaces were analysed. The average IVD height was calculated by taking the sum of the anterior and posterior heights using Dabb's method (Dabbs and Dabbs, 1990). Data was compared between SkinSuit/non-SkinSuit conditions and expressed as either means ± SD (stature and IVD height which were compared using a paired t-test) or median ± interquartile range (subjective ratings which were compared using a Friedman's test). Statistics were performed using Statistical Package for Social Sciences 24.0 (SPSS IBM, Chicago, IL, USA) with significance assumed when $p < 0.05$.

Section 4.03   Results

All subjects successfully completed both 8h conditions (with and without the SkinSuit) on the HBF without incident, with no incidental findings. Stature elongation (pre-vs. post 8h HBF) was non-significantly attenuated (0.4mm; p=0.18) when wearing the SkinSuit (1.7±0.5cm vs. 2.1±0.4cm; Figure 16).

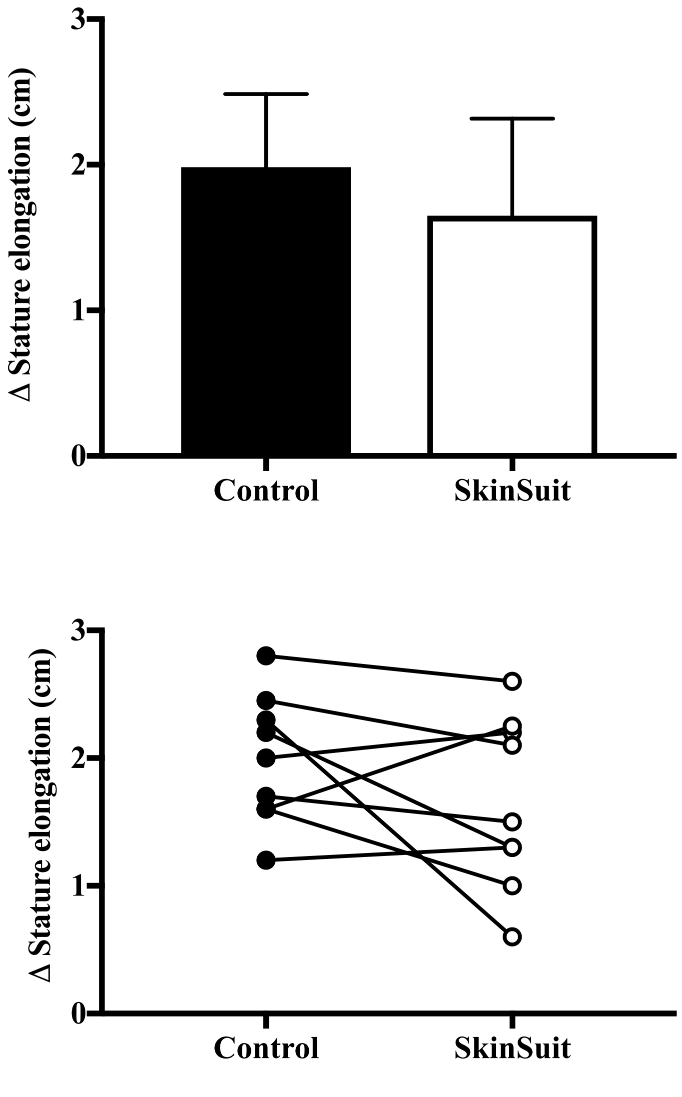
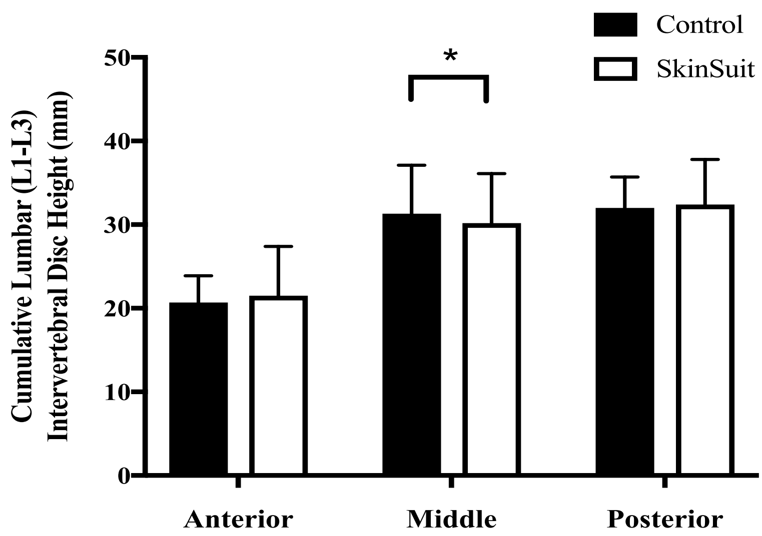


Figure 16. Top Panel –Delta (Δ) stature elongation (mean±SD) after 8h HBF compared between attires. Bottom Panel – Individual Δ stature elongation after 8h HBF compared between attires.

The average height of the lumbar IVD spaces did not significantly differ between attires, although 5 (out of 9) individuals showed lower average disc height (Figure 17; *top panel*). When split into each component (anterior, middle and posterior IVD height), the height measured in the middle of the disc space was significantly ($p<0.05$) lower (Figure 17; *bottom panel*). This was attributed to an attenuation ($p=0.032$) of L1/L2 IVD height of 1.7mm (8.5±1.3 vs 9.2±1.5mm). The reduction in IVD height measured at L1/L2 corresponds to 50% of the stature attenuation in the SkinSuit.



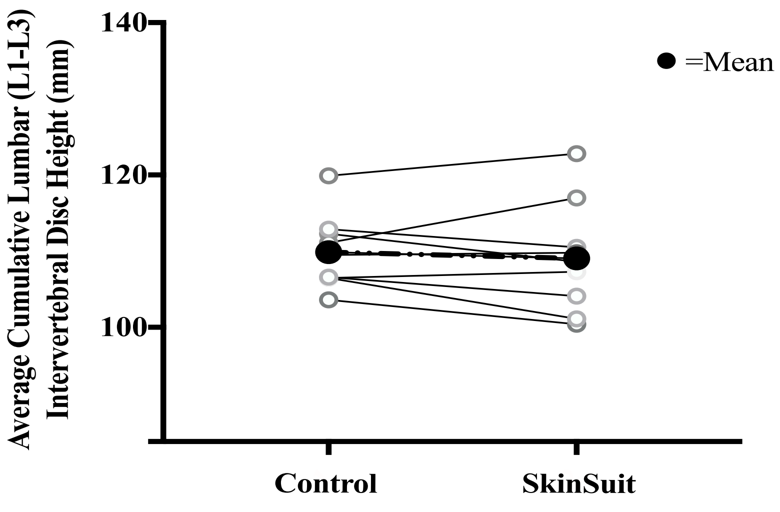


Figure 17. Top Panel –Lumbar Intervertebral disc heights (L1-L3) measured at each part of the disc (mean±SD). Bottom Panel - Individual average (Dabbs method) of cumulative lumbar IVD heights (L1-L3). * Significantly lower with the SkinSuit p<0.05.

At the end of 8h HBF, ratings of increased movement discomfort (2.5 [0-5] vs. 0.5 [0-2]) and lower body control pertaining to perceived effort with wear (2.5 [0-4] vs. 2 [0-1]) were reported with the SkinSuit. The greatest discomfort recorded was 7 by a single participant wearing the SkinSuit which was defined as "too uncomfortable to wear for 4 hours", despite the fact the subject completed the 8-hour without complaint. Whilst subjective lower back pain was not significantly different between conditions over time, it tended to be lower (p=0.11) with the SkinSuit with a considerably lower interquartile range (Figure 18).

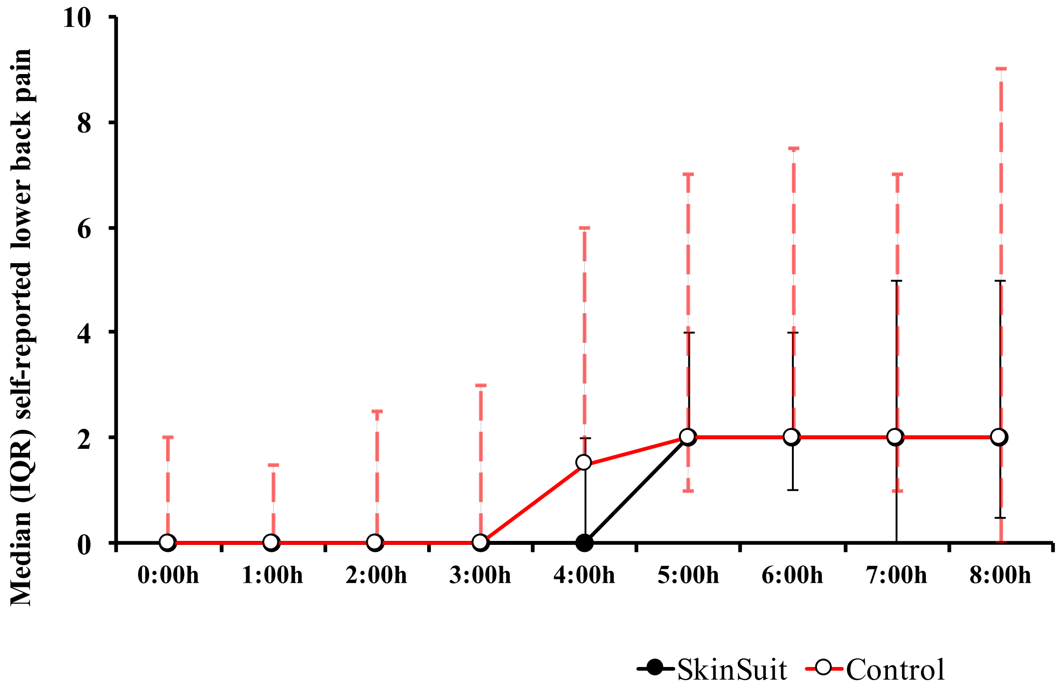


Figure 18. Rating (0-10) of lower back pain (median±interquartile range) during HBF.

Section 4.04 Discussion

The main findings of the study were that stature elongation induced by HBF (1.7-2.1cm) was reduced by approximately 20% in the Mk VI SkinSuit which imparted 0.13±0.03Gz at the foot. No difference in average IVD height was observed, though a significant decrease in the central lumbar IVD height was recorded at L1/L2 specifically. Discomfort was experienced whilst wearing the SkinSuit compared to gym clothes, though the intensity of self-reported back pain tended to be lower when wearing the SkinSuit (p=0.11). No integration issues of the Mk VI SkinSuit with HBF or DEXA imaging were reported.

Effects of SkinSuit loading on stature

The degree of stature elongation induced by HBF tended (p=0.18) to be attenuated by approximately 20% with the passive 0.13Gz loading imparted by the Mk VI SkinSuit. This 4mm reduction is similar to the ~6mm and ~5mm reduction in stature observed during a study of acute, 20-minute backpack load carriage of 0.15±0.05% bodyweight in either front-loading or back-loading configurations (Chow *et al.*, 2011). Not only was the bodyweight loading imparted in the backpack study slightly higher than the present study (0.15 vs. 0.13Gz) but also the moment of loading was different. Backpack positioning either anteriorly or posteriorly, creates a moment arm on the spine resulting in an increase in the compression and shear forces acting on the lumbar IVDs. In contrast, the SkinSuit does not favour an anterior/posterior position, it follows the curvature of the body, shoulder to foot. Also, rather than loading through an added ‘mass-effect’ donning the SkinSuit exerts tension on the elastic fibres axially that loads the body shoulder to foot. As such a strength of its design is that it is unaffected by environmental changes in the size of g (i.e. space/Mars) and offers the ability to study in-vivo, the compressive effects of loading and reloading (after unloading) upon the spine.

In the backpack study the authors state that all testing was done in the morning, whilst this would work to minimise potential individual differences in preloading expose prior to backpack loading, the specific time after rising is not stated. Elongation following 8h sleep is reported to attenuate by 84% within the first 3 hour and 45 minutes after rising (Tyrrell, Reilly and Troup, 1985). In the current study 8h HBF also commenced in the early morning soon (1-2hours) after rising to secure an afternoon scanning slot. Therefore, individual differences in both the preload during passage to the testing centre and the time between HBF unloading and participant's sleep, means the extent of elongation may have been affected, albeit more controlled than the backpack style. Future SkinSuit studies should therefore look to utilise an overnight HBF flotation, to better control preload and more appropriately follow the normal unloading/loading diurnal cycle.

Effects of SkinSuit loading on lumbar IVD height

Lumbar IVD height in the centre of the disc was significantly reduced by 1.7mm at L1/L2 by the Mk VI SkinSuit. This corresponds to approximately 40% of the attenuation in stature elongation, which suggests IVD compression may be a significant contributing factor to the reduction in gained stature elongation. However, in a study that used 50% bodyweight supine loading and found both reductions in spinal length and IVD compression, no association was found between a reduction in spinal length and either intervertebral angle or IVD height (Kimura *et al.*, 2000). This is possibly due to the low participant numbers in their study (n=8) which were not sufficient for correlations (Moinester and Gottfried, 2014). A study investigating creep loading changes in cadaveric lumbar motion segments found a reduction in height of 1.53±0.34mm after 6 hours of creep loading at a 1000N (Adams, Dolan and Hutton, 1987). This degree of height reduction is similar to that observed in this study. However, such localised reduction in IVD height at L1/L2 does not fully explain the 4mm reduction in total stature with the Mk VI SkinSuit loading, therefore other factors are likely contributing to the stature reduction. The accuracy of measurement using DEXA is also a factor to consider, whilst the minimal detectable change of 0.32mm was able to detect a significant reduction, the range between measures of central IVD height was as high as 1.4mm. Whilst this provides an indication that scans taken by DEXA can be used to inform measures of IVD space, the resolution of the IVD is such that accuracy is questionable when evaluating an intervention, therefore gold-standard imaging (i.e. MRI) should be pursued to improve measurement of IVD geometry and clarity of images.

Previous studies have reported that 40% of the stature elongation during sleep is attributed to the lumbar spine (L1-S1), through IVD expansion (Wing *et al.*, 1992). Modelling studies found that the greatest compressive and shear forces with backpack loading occurs at the L4/L5 level, with little or no effect at L2/L3 (Wettenschwiler *et al.*, 2017). However, a study using upright MRI with 10% bodyweight backpack loading found a significant reduction in the L4/L5 and L5/S1 IVD height and no change in lumbar lordosis (Shymon et al. 2014). Whereas, another backpack study observed a decrease in lumbar lordosis but an increase in thoracic kyphosis after 30 minutes (Hung-Kay Chow *et al.*, 2011). Differences could be due to variances in study design and position of subjects for imaging (supine vs. standing). Nevertheless, in future SkinSuit assessments, it will be important to capture all lumbar IVD heights along with lumbar curvature, as with the application of loading it would be expected to observe a reduction in lumbar length, through a reduction in lumbar IVDs heights and/or a change in lumbar lordosis.

In some participants, lower lumbar levels were distinguishable in the DEXA images, but only these 3 levels (L1-L4) were measurable in all participants. A strength of the DEXA imaging is it is compatible with the metallic components of the SkinSuit (and has a relatively low radiation dose compared to CT scanning), however due to its low resolution it is unable to distinguish differing spinal tissues i.e. the IVDs themselves. Also, as not all lumbar vertebrae were distinguishable, curvature changes could not be investigated as is possible with MRI (Kimura *et al.*, 2001). Combined these factors may have masked the small but important effects of the SkinSuit in loading the lumbar spine. Therefore, future studies should seek to modify the SkinSuit to be compatible with MRI, as this would facilitate differentiation of spinal tissues and clearer resolution of all vertebral levels, to determine the effects of SkinSuit loading upon the entire spine, IVDs and curvature.

Effects of SkinSuit loading on subjective measurements

Participants reported mild discomfort from wearing the Mk VI SkinSuit after having worn it for over 4h, with one individual rating it as too uncomfortable for 4 hours of wear, despite this being near the end of the 8h period of the study. Previous incarnations of the SkinSuit studied during exercise (Attias *et al.*, 2017) and parabolic flight (Green *et al.*, 2014), also recorded mild discomfort so this was not unexpected. The reasons for one individual experiencing a greater level of discomfort were due to tailoring issues, specifically the yoke (chest) fit, where the material running across and under the shoulder was rubbing the participant's skin. Improvements in the measurement process to ensure a tailor made fit for participants, and the translation of measures to fabrication is recommended potentially through using 3D scanning as opposed to manual measurement (Kendrick, 2016). Reports of back pain in combination with observations of disc swelling has been observed in spaceflight analogues, with 92% of participants in a 3-day dry immersion trial reporting back pain using a 1-10 visual analogue scale (Treffel et al. 2017). In the present study with SkinSuit loading there was a trend for a reduction in back pain intensity, the mechanism of which is unknown. This could be due to a reduction in disc swelling as observed in the reduction in central disc height, however due to the limited imaging data and low subject number this is speculative. Also, a further limitation is that scanning was performed at the end of 8h HBF, not pre-and post as stature was. Thus, an opportunity to do scanning of the IVD's pre-and post flotation would be able to determine if IVD swelling had occurred.

SkinSuit design

The Gz loading provided by the Mk VI SkinSuit in the present study is considerably lower (0.13Gz) than the ~0.7Gz static axial loading Pingvin suit (Kozlovskaya and Grigoriev, 2004; Barer, 2008), which was recently discontinued from flight use due to its low uptake, attributed to the discomfort associated with wearing, especially during exercise. The current loading is also less than the forerunner of the SkinSuit namely the GLCS that provided ~0.8Gz but was compatible with both acute aerobic (Attias et al. 2017) and strength exercise (Carvil *et al.*, 2017). However considerable redesign was required to facilitate long term wear such as the 8 hours in the present study (Green *et al.*, 2015) and proposed for Andreas Mogensen's ISS in-flight evaluation. Improvements included an enhanced webbing and padding across the shoulders to distribute loading to improve comfort, a change in material to produce a more consistent, lower loading for tolerability and an improved ability to don/doff the garment without need for additional crew assistance. At the completion of the present study, a flight suit using recommendations for improvements was being prepared for Andreas Mogensen's 10-day technology demonstration flight within which a flight version of the Mk VI SkinSuit was evaluated (Figure 19).

Figure 19. Andreas Mogensen trying out the Mk VI SkinSuit prior to launch with ESA Astronaut Alexander Gerst. Image Credit ESA.

Conclusion

The low level (0.13Gz) axial loading provided by the SkinSuit, partly attenuated HBF-induced stature elongation. Lumbar IVD height was also partly attenuated with SkinSuit wear, accounting for 40% of the attenuation in stature between conditions. This suggests effects of Mk VI SkinSuit loading upon the spine may be manifesting in other areas of the spine, in IVD geometry and regional curvatures. Further studies with MRI are warranted to comprehensively determine the effect of axial loading upon the spine, if the SkinSuit can be rendered compatible.

The Mk VI SkinSuit was successfully integrated into HBF, a novel microgravity analogue platform, although mild discomfort was associated with wear. A trend for subjective reduction in the intensity of lower back pain, compared to without the Skinsuitwas also experienced during 8h HBF, though the mechanisms behind this are unknown. The hypothesis that the passive axial loading imparted by the SkinSuit would attenuate stature elongation from 8h HBF was positively supported by the results of this pilot study, whereas the effect upon the lumbar IVDs remains to be defined (Chapter 5).

Chapter 5. Examining the effects of Mk VI SkinSuit axial loading upon stature, the spinal column and disc geometry using MRI after 8-hour hyper-buoyancy flotation

Section 5.01 Introduction

The Mk VI SkinSuit provides low-level axial loading, from shoulder to foot and is currently under investigation as a potential spaceflight countermeasure for spinal elongation. A previous study (Chapter 4) found the Mk VI SkinSuit to significantly attenuate the degree of stature elongation incurred from 8h unloading on the HBF (Carvil et al. 2016). However, the exploratory spinal imaging performed in the SkinSuit with dual x-ray absorptiometry (DEXA) was inadequate to understand the impact of the SkinSuits loading upon the spine as it only provided information on vertebral geometry, not the intervertebral discs (Chapter 4). Magnetic Resonance Imaging (MRI) facilitates greater visualisation and differentiation of the tissues that make up the spine (Wassenaar *et al.*, 2012). Therefore, the Mk VI SkinSuit has been modified by replacing ferrous metal components with other materials, to ensure MRI compatibility, facilitating broader exploratory studies.

In-vivo MRI studies on the application of loading to the spine have found reductions in stature (Chow *et al.*, 2011) and IVD height (Shymon et al. 2014). Mixed findings have been found with the application of loading on lumbar length that either decreased (Kimura *et al.*, 2000) or stayed the same (Shymon et al. 2014) or lumbar lordosis, which either increased (Kimura *et al.*, 2001), decreased (Chow et al. 2011) or did not change (Shymon et al. 2014). Differences in findings could be attributed to variations in design (i.e. loading protocols) and imaging. Two studies used backpacks to apply loading of between 10-15% bodyweight on the shoulders when upright and measured changes either with goniometry (Hung-Kay Chow *et al.*, 2011) or MRI (Shymon et al. 2014). The application of loading upright on Earth is different to that proposed in space due to the added effects of gravity acting on the spine prior to and during testing, which could preload the spine effecting intervertebral mechanics and response to loading (Schmidt et al. 2016). The application of loading supine has been done with a harness applying up to 50% bodyweight loading from shoulder to foot (Kimura *et al.*, 2001). This supine loading method has been found to provide similar results of increased anterior disc height and lumbar curvature and decreased posterior disc height, when compared with upright weight-bearing MRI in the same subjects (Lee *et al.*, 2003). This amount of loading is far greater than the designed 0.2Gz imparted by the Mk VI SkinSuit. A previous iteration of the suit, the Mk III imparted a ~0.8Gz which was too uncomfortable to wear for more than 2h's (Carvil *et al.*, 2017), despite advances in textiles and tailoring follow-on attempts at designing a comfortable high-loading garment (up to 1Gz) for long duration wear have been unsuccessful (Kendrick, 2016). Thus, further testing on the current Mk VI is recommended using an environment and design that reproduces the environment of space.

In the previous SkinSuit evaluation, Chapter 4, the effect of 8h static unloading on the HBF was investigated during the day, commencing less than 3h after participants had risen from sleep (Carvil et al. 2016). In diurnal studies of sleep, the greatest amount of elongation was observed in the first 4h of sleep with 84% lost in the first 3h 45 minutes after rising (Tyrrell, Reilly and Troup, 1985). Further studies on diurnal elongation also have shown that 40% of elongation arose from an increase in lumbar length with no change in lordosis (Wing *et al.*, 1992). Thus, evaluating countermeasure effectiveness to acute unloading could perhaps be better facilitated after 8h of unloading overnight on the HBF followed by morning imaging to closer align with studies on diurnal elongation. Additionally, as sleep involves subtle movements of the body, this could be more analogous of in-orbit operations than static supine rest as performed in the previous 8h Mk VI SkinSuit study. No significant difference in terms of stature recovery have been observed between sleeping positions i.e. supported seating, side lying and supine hyper-extension, therefore this design may provide a more analogous and less disruptive method of unloading for participants (Healey *et al.*, 2008).

The majority of spinal research pertaining to spaceflight has focussed on the lumbar spine. However, there has been increasing interest in understanding the mechanisms that might be contributing to the increased occurrence of cervical disc herniation

(Belavy *et al.*, 2016), as this is the second most reported site of herniation in astronauts (Johnston *et al.*, 2010). This may be attributed to the increased IVD swelling as observed with the lumbar IVDs on Earth during dry immersion (Treffel *et al.*, 2016) and head-down tilt (Belavý *et al.*, 2011). However, these analogues might not be suitable for assessment of the cervical spine. In dry immersion the head is out of the water and the cervical spine is loaded by the weight of the head (Navasiolava *et al.*, 2011). Whilst in HDT participants utilise their upper body in a tilted manner to read, watch T.V. etc., this may exacerbate the recruitment of neck musculature, thus contributing to observed hypertrophy of this region which is not representative of spaceflight (Belavý *et al.*, 2013). HBF therefore may be an alternative platform to study the effects of unloading and loading upon the cervical discs, therefore an exploratory study is recommended. The Mk VI SkinSuit loads shoulder to foot, as such it should not directly impact the cervical discs but due to muscle tension acting on the processes of the vertebrae it could potentially impact this region, thereby requiring investigation

An additional question from previous SkinSuit studies is whether the control condition is suitable for its evaluation. The SkinSuit is designed to impart a circumferential pressure upon the skin of approximately 10mmHg (Waldie and Newman, 2011). However, this is estimated from material studies, not in vivo observations, thus it is currently unknown as to the interactional effects of axial and circumferential loading. Exercise studies completed with the SkinSuit compared to control conditions have found it decreases the cost of exercise by reducing the work required to achieve a targeted VO_2 (Attias et al. 2017), which could be attributed to the compressive effects of the garment supporting venous return. Abdominal binding has been used in both exercise (West *et al.*, 2014) and post-surgery (Clay *et al.*, 2014) to improve abdominal compliance, with reported mixed effects upon intraabdominal pressure (IAP). In the exercise study, an increase in IAP was reported with moderate exercise (West *et al.*, 2014), presumably due to the increased muscle recruitment. Whilst no clinically relevant effect was reported in the study with abdominal binding, an average increase in IAP of 4.4cmH_2O was observed compared with control, this may be due to imbalanced groups or the static position (Clay *et al.*, 2014). Both increased IAP (Hodges *et al.*, 2005) and long duration spaceflight (Sayson *et al.*, 2015) have been associated with an increase in lumbar stiffness. This might be associated with a compensatory stabilising mechanism (Essendrop, Andersen and Schibye, 2002), as with spaceflight the extensor paraspinal muscles atrophy (Chang *et al.*, 2016), potentially causing an imbalance in spinal stabilisation and an increase in abdominal muscle activation. Therefore, until evaluation of the Mk VI SkinSuit's potential effect on IAP is characterised, an alternative control condition must be considered to differentiate the axial loading from potential circumferential effects.

As the Mk VI SkinSuit is being readied for operational evaluations on the international space station further information on its efficacy as a potential spinal countermeasure is required. The hypothesis was that the axial loading imparted by the SkinSuit from shoulder to foot would attenuate the effects of 8h HBF unloading by reducing stature, lumbar length and/or IVD height.

The aims of this pilot study were to: -

- Compare the effect of wearing the Mk VI SkinSuit in a loaded configuration throughout the testing period, with an unloaded configuration (stirrups unclasped) upon stature, spinal length and lumbar IVD height after 8h HBF unloading
- Explore if cervical and thoracic IVD height would be affected by SkinSuit loading

Section 5.02 Methods

Experimental Approach

Ethical approval was sought and approved by the King's College London ethics committee (HR-15/16-2161) and consisted of two counterbalanced conditions. Metallic components of the SkinSuit were removed and replaced with plastic materials (i.e. zips and buckles) to become MRI compatible. The main outcome measures were stature, spinal length, IVD height and lumbar lordosis, for which the repeatability of measurement (ICC), the standard error of measurement (SEM), range and minimal detectable change (MDC) was calculated for MRI parameters (Table 4).

Table 4. Intra-observer reliability, variation of measurement and minimal detectable change of MRI parameters by the radiographer.

Parameter	ICC (95% CI)	Mean	SD	SEM	Range	MDC
Anterior IVD height (mm)	0.968 (0.921-0.987)	10.3	0.42	0.07	1.3	0.21
Posterior IVD height (mm)	0.945 (0.848-0.968)	6.2	0.4	0.09	1.22	0.25
Spine length (mm)	0.998 (0.996-0.999)	204	0.6	0.02	2.1	0.5
Cobb Angle ^{o}	0.984 (0.944-0.955)	41	1.41	0.17	3.87	0.49

Participants

Six males (31±4y; 1.75±0.08m; 76.9±9.2kg) gave written informed consent to participate in the study. They were asked to abstain from vigorous exercise in the day leading up to the study, but were encouraged to undertake normal activity on each day. None reported a history of neurological, cardiorespiratory and/or psychological disorders, nor severe, chronic back pain, a discectomy or had recently sought treatment for musculoskeletal issues. Each attended a familiarisation where they were fitted for an MRI compatible SkinSuit and loading assessed using the Forceshoes (ForceShoes, Xsens, Netherlands). The average loading produced at the foot was 0.15±0.04Gz.

Protocol

Participants were requested to attend the laboratory on two separate nights no more than a month apart acting as their own controls, where they slept for 8h on the HBF followed (upon waking) by transit to an MRI Scanner (MRI Unit, St Thomas' Hospital, London) using public transport. They wore the SkinSuit in a loaded configuration in one session from the beginning of sleep to scanning and unloaded in the other, acting as their own controls (Figure 20).

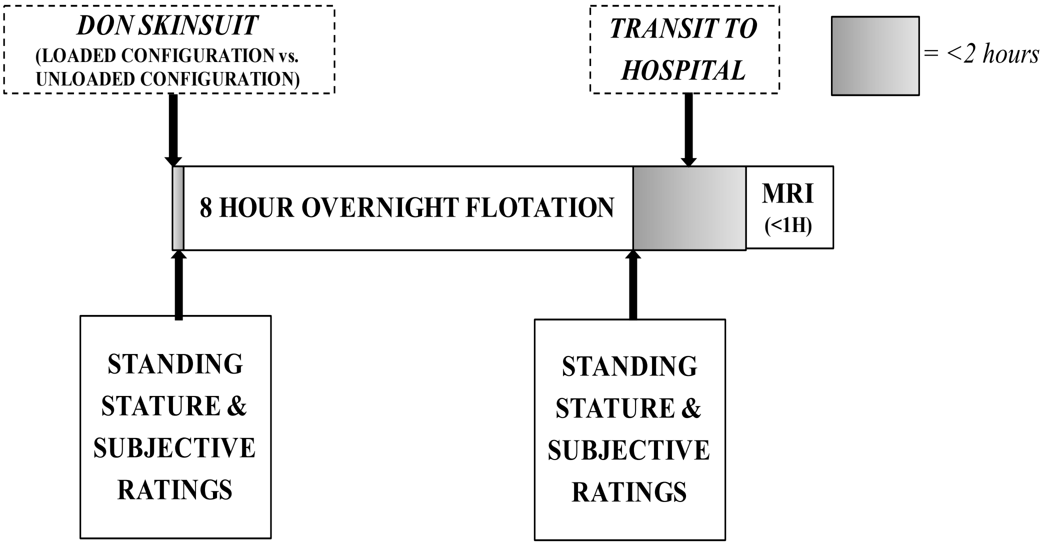


Figure 20. Schematic diagram of study protocol detailing pre-and post-sleep measurement of stature and subjective rating scales followed by transport to MRI scanning, this was repeated once when wearing the SkinSuit <u>loaded</u> and again <u>unloaded</u> on a separate night.

Upon arrival on each of the two testing sessions participants donned the SkinSuit, one time it was fastened to load the participants (loaded) and the other unfastened (unloaded - Figure 20). Standing stature (Cambridge measurements systems, UK) and subjective visual analogue scales of movement comfort (Corlett and Bishop, 1976), body control (Cooper and Harper, 1969) and back pain (Appendix) were asked before (pre) and after (post) 8h overnight HBF. After overnight HBF participants were transported to St Thomas's Hospital for MRI. Transportation from the HBF via public transport to the scanner took up to 2h depending on travel conditions, when participants arrived they rested on a reclining plinth for at least 15 minutes prior to scanning. Participants were then positioned inside the scanner by an MR technician, with a triangular pad placed under the knees. A Siemens Magnetom Aera 1.5T XMR Scanner (Siemens Healthcare GmbH, Erlangen, Germany), took six

T2 weighted sagittal slices of the whole spine, cervical to lumbar (6mm thickness, 1500/102ms repetition/echo time, 40cm field of view), parallel to the spine on coronal localisers. T2 allows more magnetization to decay before measuring the signal by altering the spin echo time, thereby fluid inside the IVD's is brighter (Figure 21). A clinician inspected the scans for pathology and interpreted them.

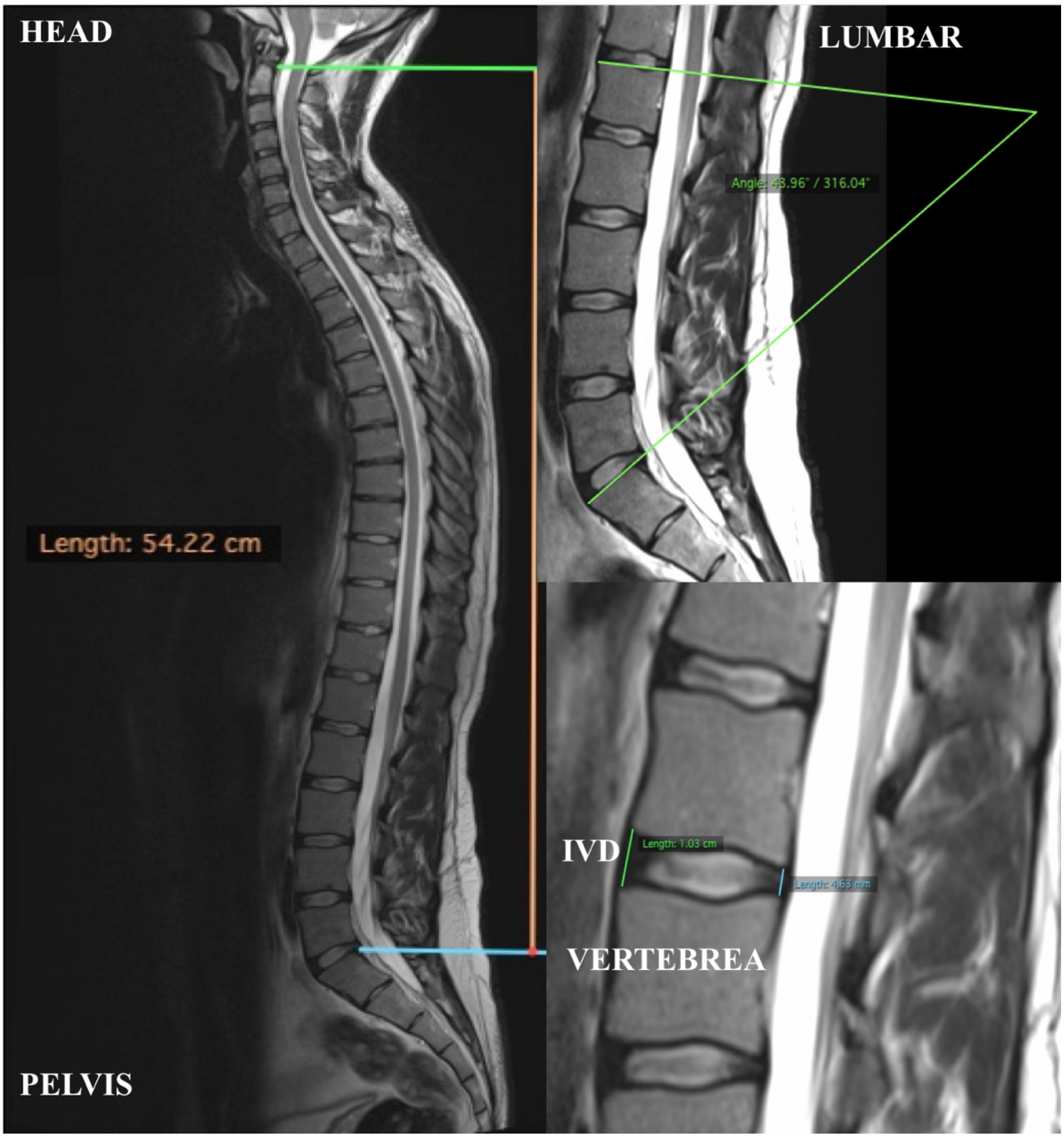


Figure 21. Image analysis of spinal length (left), curvature and IVD height (right). Image Credit – Guys and St Thomas NHS Foundation Trust.

Data analysis

Test suitability and reporting were determined by the type of data (subjective vs. objective measures) and normality through a visual check of histograms and by assessing whether the skewness and kurtosis ratio lay below or above 1.96/-1.96 (Fallowfield, Hale and Wilkinson, 2005). Data were compared between the loaded SkinSuit and unloaded Skinsuit condition and expressed as either means ± SD (stature, spinal lengths and IVD heights – t-tests) or median ± interquartile range (lumbar curvature and subjective ratings - Wilcoxon). MR images were analysed using OSIRIX (OsiriX Lite, Pixmeo Sarl, Switzerland). Spinal length was determined using the distance between horizontal lines drawn from the dorsocranial of the C2 odontoid process and S1 superior endplate. The length of the cervical spine was measured between C2 odontoid process and T1 superior endplate, thoracic T1 superior endplate to L1 superior endplate and lumbar between the L1 and S1 superior endplates. Cervical, thoracic and lumbar IVD heights were determined by measuring the distance between cranial and caudal edge both anteriorly and posteriorly. For the lumbar spine (L1-S1) Dabb's method (Dabbs and Dabbs, 1990) was also employed to calculate the average IVD heights in the lumbar spine, by averaging the anterior and posterior heights due to their larger size relative to cervical and thoracic discs. Cobb's method evaluated lumbar curvature through the angle formed between tangent lines drawn from the L1 and S1 superior endplates. Statistics were performed using Statistical Package for Social Sciences 24.0 (SPSS IBM, Chicago, IL, USA) with significance assumed when $p < 0.05$.

Section 5.03 Results

All six participants could sleep 8h overnight upon the HBF without hindrance from the SkinSuit or HBF. The following results are displayed as SkinSuit loaded vs. SkinSuit unloaded. Stature increased in both conditions after overnight HBF, a trend (p=0.18) in the loaded condition for reduction in stature was observed (1.2±0.8 vs. 2.1±0.6cm). Total spinal length (C2-S1:59.4±2.1 vs. 59.6±2.4cm) remained unchanged along with cervical (12.4±0.5 vs. 12.6±0.7cm) and thoracic (28.8±1.1 vs. 29.6±1.2cm) length. Lumbar length (L1-S1:17.8±1.0 vs. 18.1±0.8cm) was attenuated with loading (p=0.11). No significant difference (p=0.25) with SkinSuit loading was observed in lumbar curvature 40.8 [38.7-42.7] vs. 35.5 [34.4-44.4]^{o}. However, minor increases in curvature were observed in 4 participants with loading (Figure 22).

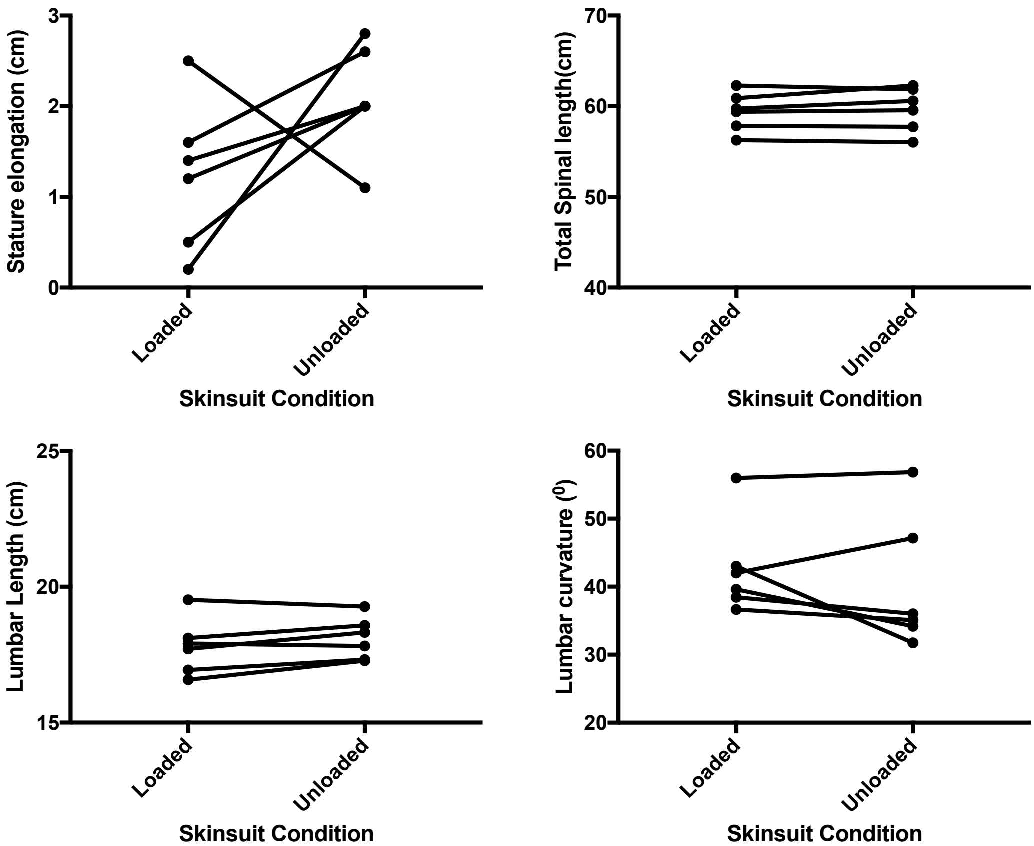


Figure 22. Individual data plotted (loaded vs. unloaded) for stature elongation post 8h HBF and MR results for total spinal and lumbar length and lumbar curvature.

There were reductions in the IVD height of several of the thoracic discs and the one cervical disc (C7/T1). A tendency (p<0.2) for an increase in the posterior height of C5/C6 with loading was observed (Table 5). Though not significant, anterior cervical IVD height was also greater in most of the cervical discs with loading.

Table 5. Anterior and posterior IVD height (mean±SD) of cervical and thoracic spine.

IVD Height (mm)	**Loaded**		**Unloaded**	
	Anterior	Posterior	Anterior	Posterior
C2/C3	3.5±1.3	3.7±0.9	3.6±0.5	3.5±0.7
C3/C4	3.6±1.1	3.4±0.7	3.1±0.6	3.4±0.7
C4/C5	3.8±1.8	3.6±0.9	3.5±0.6	3.2±0.7
C5/C6	3.9±1.0	3.5±0.6$^{\$}$ **_p=0.07_**	3.7±0.9	3.2±0.6
C6/C7	3.8±0.4	3.5±0.7	4.5±0.8	3.4±0.4
C7/T1	4.1±0.9	_2.9±0.6*_ **_p=0.04_**	3.9±0.6	_3.2±0.4_
T1/T2	3.2±0.8$^{\$}$ **_p=0.116_**	3.1±0.6	3.5±0.6	3.3±0.5
T2/T3	2.8±0.7	3.3±0.7$^{\$}$ **_p=0.17_**	2.8±0.5	3.2±0.4
T3/T4	2.6±0.5	3.0±0.6$^{\$}$ **_p=0.07_**	2.5±0.3	2.9±0.7
T4/T5	2.5±0.5	2.9±0.6$^{\$}$ **_p=0.07_**	2.6±0.5	3.0±0.4
T5/T6	2.8±0.6	3.2±0.5$^{\$}$ **_p=0.07_**	2.8±0.5	2.8±0.3
T6/T7	3.1±0.8	3.1±0.7	3.0±0.5	3.0±0.4
T7/T8	3.4±0.4	3.4±0.8	3.6±0.7	3.3±0.4
T8/T9	3.5±0.4$^{\$}$ **_p=0.11_**	_2.9±0.5_$^{\$}$ **_p=0.07_**	3.8±0.6	_3.5±0.5_
T9/T10	_3.9±0.5_$^{\$}$ **_p=0.20_**	_3.7±0.6_$^{\$}$	_4.2±0.8_	_4.1±0.8_
T10/T11	_4.1±0.8*_ **_p=0.03_**	3.9±0.6	_4.6±1.0_	3.8±0.7
T11/T12	4.6±1.0	_4.1±0.9_	5.0±1.2	_4.7±0.8_
T12/L1	5.5±2.3	_4.4±1.2_$^{\$}$ **_p=0.07_**	5.5±0.3	_5.3±1.3_

* significant difference (p<0.05) between loading conditions and $^{\$}$ a trend (p<0.2).

For Lumbar IVDs, overall there was no significant differences in the average or anterior/posterior disc heights. However, at the lower levels (L3/L4-L5/S1) a minor reduction in average IVD height of 0.2-0.3mm was seen, with a trend ($p<0.2$) for attenuated posterior height at the L1/L2, L3/L4 and L4/L5 IVDs in the loaded condition (Figure 23).

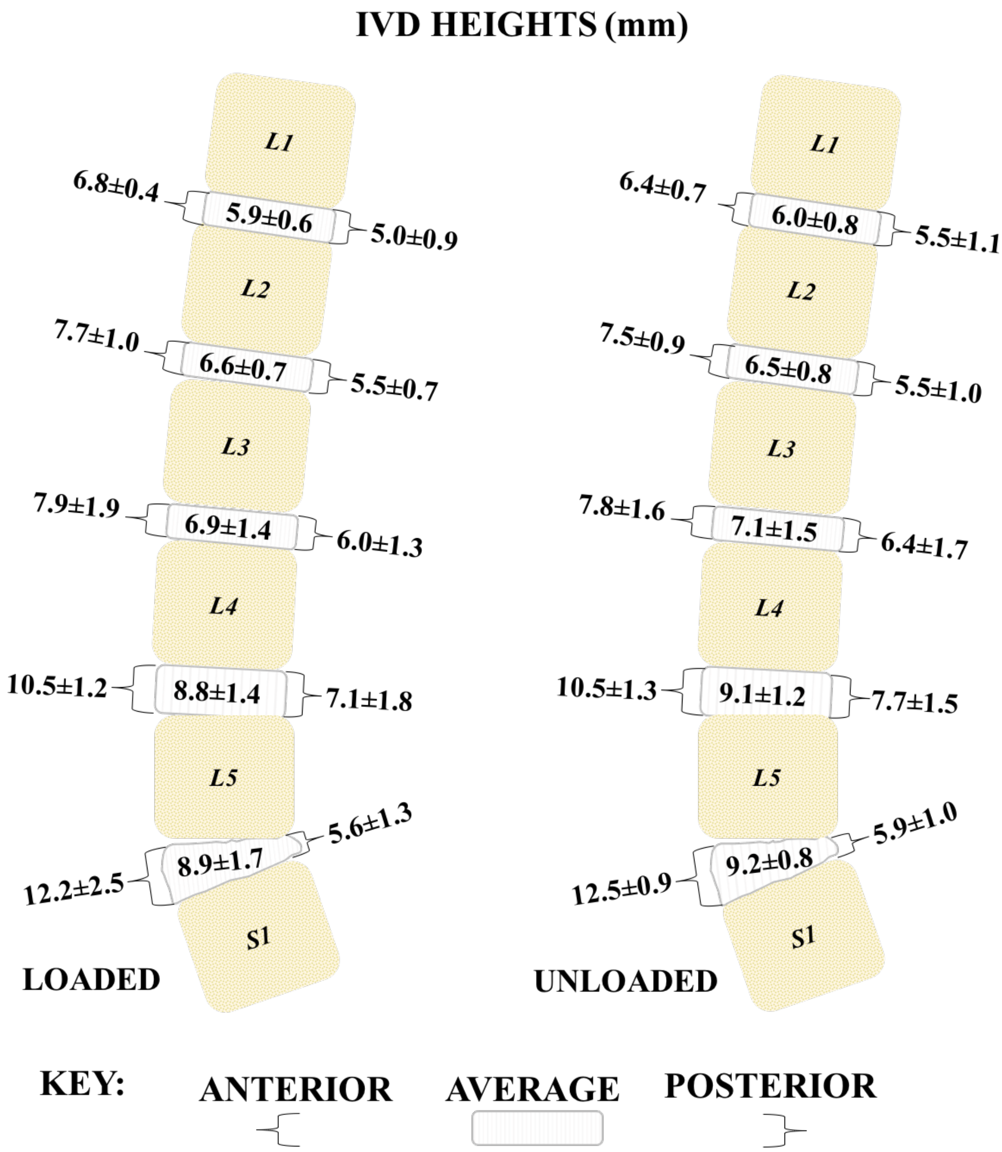


Figure 23. Anterior, posterior and the average ([anterior + posterior]/2) IVD heights (mean±SD) with SkinSuit loaded (left) and SkinSuit unloaded (right). * trend observed ($p<0.2$).

There was no observed difference between loaded and unloaded conditions following 8h overnight SkinSuit wear in the ratings of movement discomfort (4 [4] vs. 4 [3.25-4]), body control (4 [4] vs. 3.5 [3-4]) or lower back discomfort [0 (0-0.75) vs 0.75 (0-1.8)].

Section 5.04 Discussion

The present study investigated the effects of axial loading imparted by the SkinSuit upon stature elongation, spinal length and IVD height after 8h overnight HBF. The main findings were a trend for a reduction in stature elongation and lumbar length with axial loading, compared with the unloaded condition, following 8h HBF. A minor increase in curvature was observed in four of the six participants, though this was not statistically significant. A tendency for a reduction of height in several lumbar and thoracic IVDs was observed. Whilst no significant cervical IVD height changes were found, there is a potential indication of IVD expansion in this region. The hypothesis that SkinSuit loading would attenuate stature, lumbar length and IVD height is partly supported by these findings. However, due to the low subject number in this pilot study conclusions are speculative.

Measurements of displacement (stature, length and IVD height)

Following 8h HBF significant stature elongation was experienced in both the unloaded and unloaded condition. With the loading condition, there was a tendency ($p<0.2$) for an attenuation in stature elongation. The degree of stature elongation in the control condition (unloaded) in the present study vs. the control condition in the previous 8h HBF SkinSuit experiment (Chapter 4) was similar (2.1±0.6 vs. 2.1±0.4cm). With axial loading, there was a greater attenuation of stature elongation, in the present study vs. the previous (Chapter 4) 8h Skinsuit loaded trial (1.2±0.7 vs. 1.7±0.5cm; (Carvil et al. 2016). One participant (out of 6) experienced greater reduction in stature in the unloaded condition, therefore results were not significant. Reasons for this are unclear, but could be due to loosening of stirrups during loaded conditions, excessive non-disclosed preloading (e.g. weightlifting) prior to the loading condition effecting IVD diffusion (Arun *et al.*, 2009) exacerbating unloading or measurement error. Refinements in both protocol to avoid stirrup loosening, participant information and increasing subject numbers are recommended in future studies.

Increases in stature with unloading (and spaceflight) have been attributed to an expansion of IVDs and a reduction in spinal curvature (Styf *et al.*, 1997). In a study that sought to break down contributing elements to stature elongation, 40% was attributed to changes at the lumbar level, 40% at the thoracic with 20% miscellaneous either through soft tissue swelling (i.e. heel pads) (Foreman and Linge, 1989) or influences from cervical discs (Wing *et al.*, 1992). In this study, total spinal length was non-significantly ($p>0.2$) attenuated by 2mm with 8h+ SkinSuit loading, however measurements performed region by region observed a trend ($p<0.2$) for a reduction in lumbar length (-3mm), with a nonsignificant ($p>0.2$) attenuation of thoracic (-8mm) and cervical length (-2mm). All spinal regions are likely contributing to the overall reduction in stature, with the greatest influence from the thoracic and lumbar lengths as observed by Wing and colleagues (Wing *et al.*, 1992). However, it is important to note that due to the low subject numbers in this present, pilot study, there is an increase chance of making a type 1 error. Thus, length measures alone are not sufficient to support an effect of SkinSuit loading on spinal elongation as these would be influenced by differences between intervertebral levels.

In the lumbar IVDs a trend for a reduction in posterior IVD height was observed with loading at three intervertebral levels (L1/2, 3/4, 4/5) of 0.5, 0.4 and 0.6mm respectively, which is greater than the MDC of 0.25mm. A reduction in posterior lumbar IVD was also observed in a study which applied 50% bodyweight loading supine via a harness for 30 minutes loading (Lee *et al.*, 2003). In that study results for L1/L2 were not included for undisclosed reasons, presumably as they compared both supine loading with upright kneeling where the field of view was insufficient to capture this level. However, for L3/4, L4/5 higher reductions in IVD height of 0.6mm and 1.3mm respectively were reported than that observed in the present study, suggesting the intensity of the loading compresses the disc more than the duration of loading. A study using 100%BW loading supine for 10 minutes in-vivo also found a significant reduction of 0.4mm in L4/L5 (Wisleder, 1999). This is less than the 50% harness study (Lee *et al.*, 2003) but slightly more than the present study for which there are two reasons. The first is measurement differences, the author (Wisleder, 1999) measured the change in the distance between the centroids (geometric centre) of L4/L5 with loading and unloading. In the present study, the closest measure to this was the calculated average where a reduction at L4/L5 was 0.3mm was observed. This method of measurement could be considered in future studies to improve standardisation of measurement. Secondly, whilst the loading is higher, the duration is the shortest. In a study of 50% bodyweight loading on solute transport into the IVD, it was found that after 4.5 hours of continued loading nutrient transport into the disc is impaired potentially accelerating disc degeneration (Arun *et al.*, 2009). Loading is important to IVD remodelling and cellular matrix integrity. Rodent exercise studies have shown that with repeated bouts of exercise for 50 minutes cellular proliferation in the extracellular matrix of the outer annulus increases (Brisby *et al.*, 2010; Sasaki *et al.*, 2012), where with prolonged microgravity and unloading apoptosis pathways are upregulated (Jin *et al.*, 2013). Thus, a balance must be sought and explored further for spaceflight countermeasures where loading and duration of wear are optimised.

Measurements of the cervical discs in response to load and unloading has not received the same attention as lumbar IVDs, despite this region being identified as a high-risk site for herniation in astronauts. No current in-flight data from space has been collected on the IVDs though use of a cervical and lumbar ultrasound protocol has been developed and tested in space to image anterior disc heights (Marshburn *et al.*, 2014b). Data from one subject in a parabolic flight used a fixed collar to take measurements of anterior IVD height in the cervical region, whilst the data were noisy, they did measure a disc response to acute load and unloading of between 2mm in-flight (Buckland, 2011). In the present study, minor differences in IVD height between loading conditions were observed, with a significant decrease in the posterior height of 0.3mm at C7/T1. The SkinSuit loads shoulder to foot, as such it applies pressure across the shoulders that could potentially increase muscle tension. Pilot helmets weighing 1.5kg, have been found to increase muscle activity in the cervical erector spinae and sternocleidomastoid groups by 5.4 and 2.4% of maximal voluntary contraction activity respectively when worn (Sovelius, 2014). The increased loading of the SkinSuit might therefore be acting in a similar manner to increase the muscle activation in this region, that could then be affecting cervical load/unloading responses. As this is the first in-vivo study to investigate a spinal countermeasures effects opon the cervical spine, it is recommended to include in further testing. Firstly, on the effects of the SkinSuit on IVD height over time, as Chapter 3 observed a gradual stature elongation on the HBF, but also the prolonged effect of unloading on the cervical spine to better elucidate the mechanisms for post spaceflight injury.

The differences in displacement measures (length, IVD height) do support the notion that the application of loading induces a compression on the IVDs. However, as the spine is curved, height measurements alone in-vivo may alone be insufficient.

Lumbar curvature

With 8h of SkinSuit loading lumbar curvature was on average higher by 5^{o} as 4 of the 6 participants had increase curvature with loading, though this was not significant it is higher than the MDC of 0.49^{o} and may bare some clinical significance. It might also be due to the lower level of loading imparted of 0.15Gz. In a study comparing an axial loading harness and an upright position on the lumbar spine, lumbar curvature increased by 14.1^{o} after 30 minutes of 50% bodyweight loading (from the chest to the feet via harness), whilst after 30 minutes of upright kneeling curvature increased by 11.5^{o} (Lee *et al.*, 2003). Another study with additional 10% bodyweight for 10 minutes when upright using weighted backpacks did not see an increase in the lumbar curvature compared to normal upright, though it is interesting to note that neither was there a difference between supine and standing in their study which might be due to the brief exposure to loading of 10 minutes (Shymon et al. 2014). That study reported that whilst an hour of loading would have been optimal due to the acute effect of loading upon the spine, they incorrectly base and cite this effect of loading in their paper as an 80% change in the spine in the first hour after loading, not 50% which the original paper reports (Lee *et al.*, 2003) and others report as 54% with 1h and 84% closer to 4h (Tyrrell, Reilly and Troup, 1985). In their study, 30 minutes were chosen not 1h because participants could not tolerate kneeling for more than 30 minutes. Whilst the results from the current study were not significant, they warrant further investigation as the ability to impart loading that is comfortable for long periods of wear could provide insight both into countermeasure development for spaceflight but also the effects of loading upon the spine. The reported studies utilised both supine and upright MR which alter both the amount of loading and muscle activity on the spine, thus in order to further investigate the effect of the SkinSuit upon the spinal structure future studies should look to compare the effect of the SkinSuit in both a supine and upright position.

A study into the application of a backpack for 30 minutes at 10% bodyweight resulted in a significant ($p<0.05$) increase in the cervical curvature (lordosis) of 5.4% (Hung-Kay Chow *et al.*, 2011). An increase in this angle at the neck has been attributed to an increase in rounding of the shoulders and forward head leaning in children who carry a 10-15% bodyweight backpack (Mo *et al.*, 2013). Cervical unloading can also be accomplished via traction which has been used as a proposed method of reducing neck pain (Chumbley *et al.*, 2016). It acts through secure fitting of a neck wedge to a sliding platform that applies a controlled traction that pulls the head away from the shoulders. No neck discomfort was reported in either 8h SkinSuit study compared to the unloaded trials in Chapter 3. Whether there is a pulling force on the cervical spine, due to loading at the shoulders with the SkinSuit is unclear. Further imaging investigations of the cervical IVD's are recommended into the prolonged unloading effects to better understand their response to unloading, but also interactions with SkinSuit reloading.

SkinSuit design

The partial axial loading imparted by the SkinSuit in the present study was 0.15±0.04Gz, less than the designed 0.2Gz. Previous studies have also found that the measured axial loading imparted (0.13Gz) was less than that of the design (Chapter 4) Each SkinSuit is tailor-made to a participant's dimensions, as such fluctuations in weight and body proportions, which can occur through adaptions to stimulus (e.g. exercise) and environmental changes such as fluid shifts in spaceflight, could affect loading anchoring (Kendrick, 2016). With the limited number of SkinSuits available, others of similar dimensions could wear one if they matched the characteristics of the SkinSuit, however due to suit-user interface and anchoring, variations in loading could still exist due to insufficient stretch. Thus, without a feasible, deployable method for real-time monitoring of SkinSuit loading, there may remain a limitation with this form of axial loading technology.

In the study investigating the use of a harness to load the spine the authors note that whilst the harness was not compressing the abdominal cavity (but rather the chest so as not to effect IAP), IAP was not measured (Shymon et al. 2014). Whilst abdominal binding has been demonstrated not to affect lung function and IAP rise (Clay *et al.*, 2014), the effect of combined circumferential and axial loading could have affected their results. Parallel to the present study an investigation upon the SkinSuit's effects upon the IAP was undertaken with both SkinSuit loading, unloading and control (Figure 24). No significant difference between conditions during resting positions were observed, the only significant changes in IAP between conditions was seen with cycling with increased IAP with SkinSuit wear. This could be attributed to the increase muscle recruitment with exercise driving IAP increases (West *et al.*, 2014) coupled with the increase in workload imparted by overcoming the elastic resistance of the SkinSuit. This has been reported in previous SkinSuit exercise assessments (Attias *et al.*, 2017). Thus, during resting assessments it is concluded that the SkinSuit has no significant effect upon IAP, however further investigation during SkinSuit coupled exercise should be explored to determine if during exercise the SkinSuit could be utilised to provide increased spinal stability in instances where this is desired. Future work should thus firstly explore how the axial loading imparted by the SkinSuit effects lumbar spinal stability during passive movement.

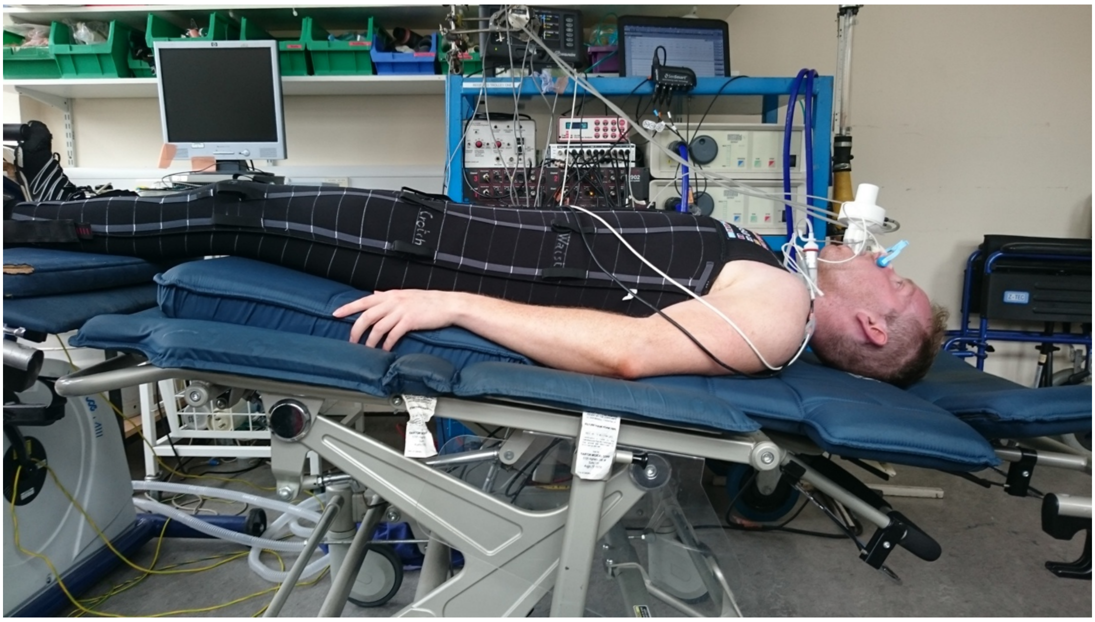


Figure 24. Parallel investigation into the effects of SkinSuit wear on intraabdominal pressure and breathing mechanics in passive (including HDT) and active situations. Image Credit King's College London, King's College Hospital and ESA.

Additional limitations and future recommendations

It has been reported that 84% of the elongation is lost within the first 3 hour 45 minutes after rising (Tyrrell, Reilly and Troup, 1985). A major limitation in the present study was the time to MR scanning and the transport to the scanner. The study took place during a time of MR scanner decommissioning for the establishment of a new clinical centre on campus, as such in order to undertake MRI at the time participants had to be transported to another hospital in the health partnership. The distance meant that there was considerable time and variation due to transport before scanning to distort potential findings. Whilst participants were placed recumbent on arrival prior to scanning significant distortion of the results could have occurred. Whilst up to several days between landing from space and spinal scanning is common in space studies due to scheduling commitments and crew safety (Sayson *et al.*, 2015), future research on the SkinSuit should seek to minimise this confounding variable. As further spaceflight, operational evaluations of the SkinSuit are planned, following successful integration into Andreas Mogensen's mission (Figure 25), follow-up investigations are recommended to understand how axial reloading effects the lumbar spine.

Figure 25. Andreas Mogensen wearing the Mk VI SkinSuit on the International Space Station during an in-flight cycling integration. Image Credit ESA/NASA.

The position of the participants on the HBF whilst they sleep was not controlled as individuals move during their sleep several times. To control this would be to negate the small movements and muscle contractions associated with sleep which could provide a more ecologically valid situation to spaceflight, rather than strict immobilisation, as astronauts move in space. A study on different unloading positions with stature recovery found similar degrees of stature recovery between supported seating, side lying and supine hyper-extension, therefore the sleeping position should not affect the degree of unloading experienced (Healey *et al.*, 2008), but also might provide greater realistic comparability with spaceflight in terms of muscle activation/movement as opposed to an imposed static position. However, it could influence the degree of loading imparted by the SkinSuit in a flexed position thereby reducing the loading, therefore an ability to track in real-time wirelessly both the loading and degree of spinal change during these positions whilst the participant rests would be advantageous and is currently being investigated (Stoppa, 2016).

Conclusion

This pilot study supports previous findings in Chapter 4 that the Mk VI SkinSuit is able to attenuate stature elongation induced from 8h HBF. MRI was successfully integrated with the SkinSuit. A lower lumbar spinal length was recorded with SkinSuit loading. However, significant lumbar IVD compression and/or preservation of lumbar lordosis was not observed compared to control. Whether the SkinSuit is effective at re-compressing an elongated spine, and if so by what mechanism, remains to be determined (Chapter 6 and 7).

Chapter 6. The effect of 4-hour SkinSuit induced partial axial reloading upon stature elongation and anterior intervertebral disc height as assessed by ultrasound after 8-hour hyper-buoyancy flotation

Section 6.01 Introduction

The MK VI SkinSuit is a proposed countermeasure for stature and spinal elongation induced by the microgravity environment, by reintroducing an axial load to the body, shoulder to foot (Green et al. 2015; Waldie & Newman 2011). Previous SkinSuit studies of this thesis (Chapter 4 and 5) have utilised hyper-buoyancy flotation to unload the body and compared the effect of wearing the SkinSuit in a loaded configuration to a control condition, either utilising gym clothes (Chapter 4) or the SkinSuit in an unloaded configuration (Chapter 5). However, this protocol design may not offer an optimum perspective of evaluating this countermeasure.

The SkinSuit is to be donned in space, at a time in the mission when astronauts would have been without 1G loading for up to several days. Thus the SkinSuit would be reloading an already unloaded spine, a subtle difference to the way previous investigations (Chapter 4/5) have evaluated the effect of the SkinSuit (Carvil et al. 2016). To address this issue, the effects of reloading with the SkinSuit upon the spine need to be studied after a suitable period of unloading. Eight hours of HBF unloading has been found to induce significant stature elongation in excess of other spaceflight analogues and that documented following sleep (Styf et al. 2001; Tyrrell et al. 1985) (detailed further in Chapter 3). Eight hours of bedrest has also been used to assess the diurnal effects of load/unloading on the spine (Ledsome et al. 1996) and stature (Tyrrell, Reilly and Troup, 1985), with assessment of stature showing that much of height gained (85%) through unloading is lost after 4 hours of loading at 1G, though the proportion attributed to the lumbar spine was not determined. Stereoscopic photography has been used to investigate the changes in the lumbar spine after 8h bedrest. Of the 16mm stature elongation induced, 40%

was attributed to the lumbar spine with a suggested 1.6mm average swelling at each intervertebral level after 8 hours of bedrest (Wing *et al.*, 1992). However, this method does not directly measure IVD height thus results are an estimation of average lumbar IVD swelling. Therefore, an imaging modality is required that can be readily employed to take multiple measures over time.

A challenge that exists with spaceflight and some analogue models is the degree to which the participant is exposed to factors that might affect the elongation process in transport to appropriate imaging. In astronaut studies can take several days from landing before imaging is performed (Sayson *et al.*, 2015). Also in the previous SkinSuit study, it took several hours before the participant could be scanned after coming off the HBF (Chapter 5). A diurnal study investigated how the lumbar spine elongated after sleep by using ultrasound, a portable method of imaging the spine. They observed an increase in the distance between the L1-L4 transverse process of 5.3mm following 8h bed-rest (Ledsome et al. 1996). A posterior approach provides a measure of lumbar length but does not permit clear visualisation of the IVDs. Ultrasound has been used to image the anterior spaces of both the cervical and lumbar regions in extreme environments (Dulchavsky *et al.*, 2002) including on the ISS where the cervical and lumbar IVD's were visualised, but not measured (Marshburn *et al.*, 2014a). For that mission, a learning tool was developed to assist in the probe placement for image acquisition. However, no ground analogue studies have been published using this protocol, nor were any data reported on height changes with ultrasound.

Despite this 'new height' of ultrasound in space and the high prevalence of hernias in the cervical spine (Johnston *et al.*, 2010; Marshburn *et al.*, 2014b), little information exists on how the cervical discs are effected by load/unloading. In the stereoscopic photography study of bed-rest induced elongation, 20% was attributed to miscellaneous sources including the cervical spine (Wing *et al.*, 1992). In a 60-day bedrest study no significance changes were observed in cervical disc height but there was a hypertrophy of the cervical musculature, which was potentially due to the head down orientation of the participants (Belavý *et al.*, 2013). However, repeat measures were taken at the start and 25 days into the study thus acute effects of unloading are unknown. Furthermore, in the latest SkinSuit study using MRI, a significant decrease in the posterior height of C7/T1 of 1.3mm was observed no changes in length were. As the SkinSuit loads from shoulder to foot further data on the potential interactions of the SkinSuit with the cervical discs under reloading is recommended, coupled with the study of acute unloading effects using a supine microgravity analogue.

As the SkinSuit is to be used in space, further information using the existing NASA protocol would provide information on compatibility of ultrasound with the SkinSuit. Therefore, the hypothesis was that reloading with the SkinSuit would attenuate the effects of unloading on stature and lumbar anterior IVD height, with no effect on cervical IVD height.

The aims of this pilot study were to:

1) Investigate how 8h unloading and 4h reloading with the SkinSuit during HBF would affect elongation
2) Evaluate the use of in-flight NASA ultrasound protocol to assess anterior IVD height

Section 6.02 Methods

Experimental approach

Approval for the study was sought and given by the King's College London ethics committee (HR-15/16-2161) which consisted of a single testing session. Mk VI SkinSuits constructed from the previous study (Chapter 5) were utilised for this pilot study. The main outcome measures were stature as performed in Chapters 3-5 and anterior IVD height of the cervical and lumbar spine undertaken with ultrasound. The repeatability of measurement (ICC), the standard error of measurement (SEM), range and minimal detectable change (MDC) was determined for the ultrasound measure of 32 anterior IVD heights (16 lumbar, 16 cervical) taken in the same day (Table 6). All ultrasound measures were taken and measured by the same operator.

Table 6. Intra-observer reliability, variation of measurement and minimal detectable change of ultrasound parameters by the author.

IVD height	ICC (95% CI)	Mean (mm)	SD (mm)	SEM (mm)	Range (mm)	MDC (mm)
Anterior Cervical IVD space	0.997 (0.994-0.998)	4.5	0.08	0.004	0.3	0.01
Anterior Lumbar IVD Space	0.997 (0.994-0.998)	11.6	0.1	0.006	0.5	0.02

Participants

Eight male participants gave written informed consent to partake in the study (27±7y; 1.78±0.07m; 70.6±10.4kg). Each attended a familiarisation session as before, where those who had not previously participated in a SkinSuit study were measured for a SkinSuit and loading assessed as in Chapter 4 using the ForceShoes (ForceShoes, Xsens, Netherlands). The average loading produced at the foot was 0.17±0.04Gz, so participants were reloaded with an average 0.17Gz during the 4h period on the HBF.

Protocol

Participants attended the laboratory in the evening where they slept for 8h on the HBF followed (upon waking) by donning the SkinSuit and returning to the HBF for a further 4h. Subjective visual analogue scales including movement comfort (Corlett and Bishop, 1976) body control (Cooper and Harper, 1969) and back pain, along with standing stature measurements were recorded before and after the 8h overnight unloading on the HBF using stadiometry (Cambridge measurements systems, UK). Upon waking and donning the SkinSuit, stature was measured again at the beginning (0h) and end (4h) of the reloading period with the SkinSuit (Figure 26).

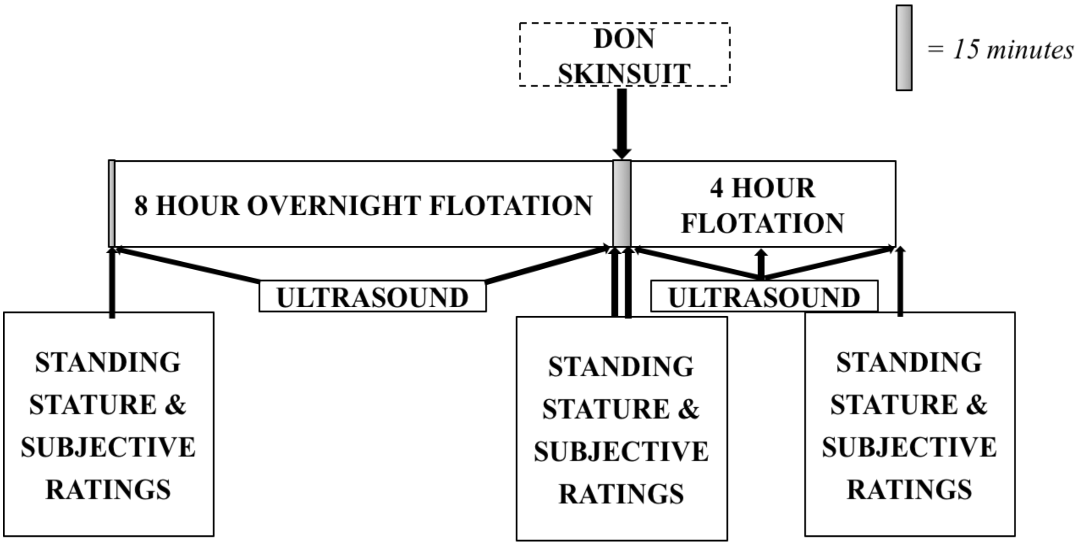


Figure 26. Schematic diagram of study protocol detailing when stature, subjective rating scales and ultrasound measurements were taken.

Ultrasound was performed using a Sonocite X-PORTE (Sonocite FujiFilm, Bedford, UK) whilst participants were on the HBF, imaging the anterior IVD heights of the cervical spine (C4/C5 - C7/T1) and lumbar spine (L2/L3 - L5/S1) laterally. This was done at the start (0h) and end of sleep (8h; unloading period) and the start (0h), middle (2h) and end (4h) of the reloading period). A 12-4 MHz linear array probe at 6cm depth was used for imaging the cervical spine, parallel to the right of the oesophagus starting just above the manubrium and running cranially up (Marshburn *et al.*, 2014b). The manubrium is used as a reference for both SkinSuit material development but also serves as a reference for T1. For the Lumbar spine, a 5-2 MHz curvilinear array probe was positioned sagittal on the midline of the abdomen with the bisection of the aorta at L4 used as the first reference marker and the sacral shelf at L5/S1 the other. Training was provided by a sonographer at St Thomas Hospital, with a total of 20 hours training prior to this study. A NASA training tool for ISS crew was used to assist in the method of obtaining images and to guide the scanning planes (Marshburn *et al.*, 2014a). Images were checked by a sonographer for marker placement and analysed by this author using the SonoCite on-board 2-point length measure feature (Figure 27).

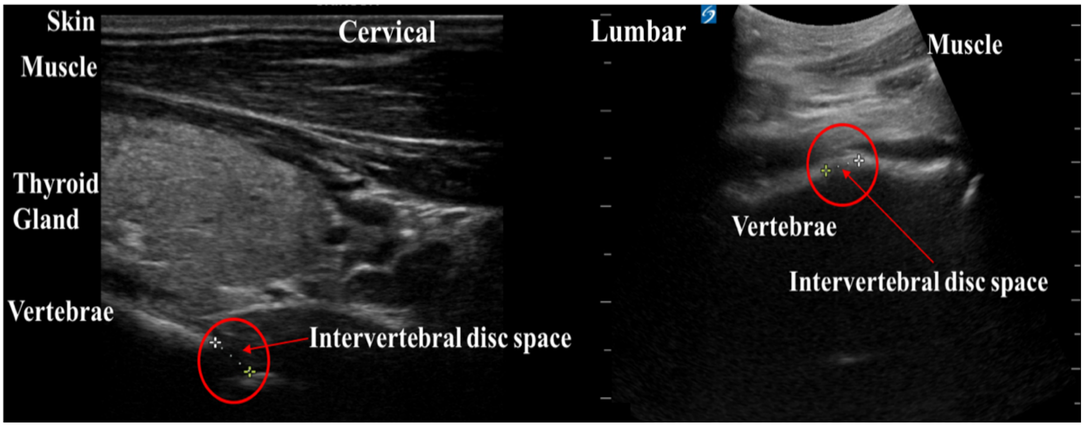


Figure 27. Acquired images of the cervical (left) and lumbar (right) disc spaces and placement of markers for anterior IVD height. Image Credit - King's College London.

Data analysis

The statistical test was determined by assessing normality of data with a visual check of histograms followed by checking if the skewness and kurtosis ratio lay below or above 1.96/-1.96 (Fallowfield, Hale and Wilkinson, 2005). Data were compared between time points and expressed as either means ± SD (stature and IVD height – t-test) or median ± interquartile range (subjective ratings - Wilcoxon). Ultrasound images were analysed using the distance between the superior and inferior anterior edges of the vertebral bodies to calculate disc height (Figure 27). The average of two measures were taken using the SonoCite on-board 2-point length measure feature by the researcher in real-time, if the difference between measurements was greater than 5% on the scan, both measurements were repeated. Statistics were performed using Statistical Package for Social Sciences 24.0 (SPSS IBM, Chicago, IL, USA) with significance assumed when $p < 0.05$.

Section 6.03 Results

All participants successfully completed overnight unloading and donned the SkinSuit in the morning without hindrance or incident. Stature was significantly ($p<0.0001$) increased after overnight sleep (177.1±7.5 vs. 179.2±7.7cm). Upon donning the SkinSuit standing stature was significantly ($p<0.0001$) reduced (179.2±7.7 Vs. 178.2±7.8cm). Following a further 4h of HBF with SkinSuit reloading, there was only marginal (0.3mm) stature elongation (178.5±7.7cm; $p=0.09$).

There was a significant ($p<0.05$) increase in three of the cervical disc heights (C4/C5, C6/C7 and C7/T1) after unloading (sleep), with no further significant differences or individual trends observed after SkinSuit reloading (Table 10; Figure 28).

Table 7. Anterior IVD height (mean±SD) of the cervical spine as measured by ultrasound.

Cervical Anterior IVD Height (mm)	Sleep (Unloaded)		SkinSuit (Reloaded)		
	0h	8h	0h	2h	4h
C4/C5 (n=7)	3.3±0.6	4.2±1.1*	3.9±0.9	3.9±0.6	3.9±0.9
C5/C6 (n=8)	4.1±1.0	4.1±0.9	4.1±1.2	4.3±0.9	4.3±0.7
C6/C7 (n=8)	3.9±0.7	4.8±0.8*	4.5±0.4	4.7±0.7	4.6±0.6
C6/T1 (n=7)	3.8±0.6	4.3±0.7*	4.3±0.7	3.9±0.6	3.9±0.6

* Indicates significant difference ($p<0.05$) after 8h unloading.

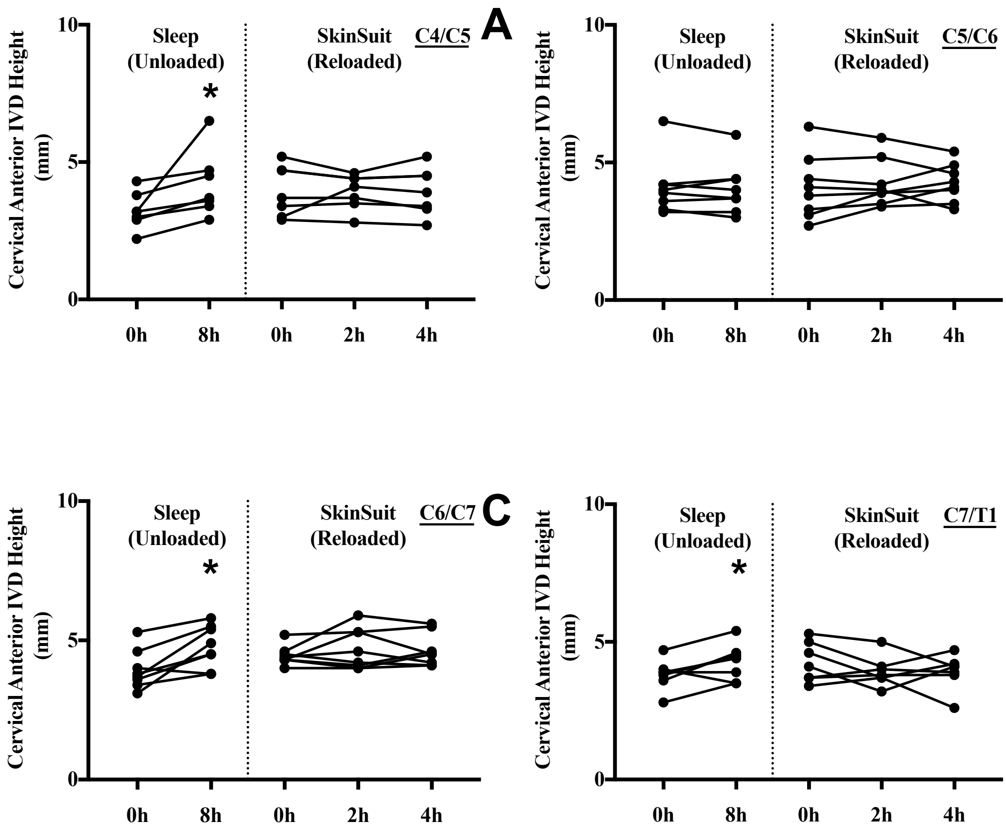


Figure 28. Individual plots of the four cervical IVD anterior height measurements taken pre-and post 8h HBF, followed by 4h SkinSuit reloading on the HBF. * Indicates significant difference (p<0.05) after 8h unloading. Panel A = C4/C5 IVD, Panel B = C5/C6 IVD, Panel C = C6/C7 IVD, Panel D = C7/T1 IVD.

After unloading the L5/S1 anterior disc height was significantly increased, and upon donning the SkinSuit at 0h of reloading it was decreased coupled with an increase in the anterior height of the L2/L3 and L4/L5 discs (Table 11), though there are substantial individual differences (Figure 29).

Table 8. Anterior IVD height (mean±SD) of the Lumbar spine as measured by ultrasound

Lumbar Anterior IVD Height (mm)	Sleep (Unloaded)		SkinSuit (Reloaded)		
	0h	8h	0h	2h	4h
L2/L3 (n=7)	8.9±1.7	9.1±0.9	10.1±1.1 §	9.1±1.7	9.0±1.4
L3/L4 (n=8)	9.8±2.7	10.1±1.7	9.9±1.8	10.4±0.8	10.1±1.8
L4/L5 (n=8)	11.3±1.1	11.7±1.3	12.2±1.6 §	12.1±01.7	12.4±1.6
L5/S1 (n=6)	9.0±2.6	11.0±1.3*	10.6±1.6 §	10.4±1.6	10.1±1.4

* Indicates significant difference ($p<0.05$) after 8h unloading, whilst § denotes significant difference between 8h unloading and donning the SkinSuit at 0h.

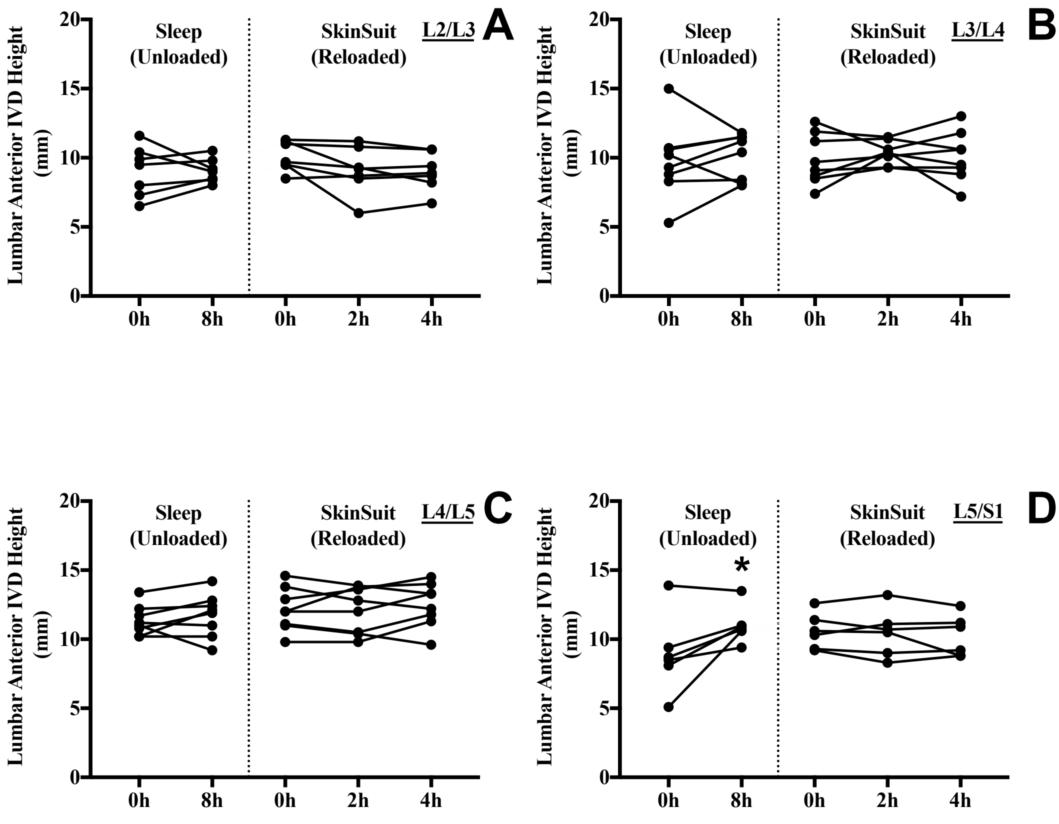


Figure 29. Individual plots of the four lumbar IVD anterior height measurements taken pre-and post 8h HBF, followed by 4h SkinSuit reloading on the HBF. * Indicates significant difference (p<0.05) after 8h unloading. Panel A = L2/L3 IVD, Panel B = L3/L4 IVD, Panel C = L4/L5 IVD, Panel D = L5/S1 IVD.

Following 8h HBF sleep in sleeping attire, two individuals reported very mild back pain (0 [0-1.4]). Upon donning the SkinSuit this dissipated (0 [0-0.4]), no further reports of back pain were reported during this time. Compared with sleeping attire in the morning, donning the SkinSuit significantly increased the degree of movement discomfort (2 [1.8-2.4] vs. 5 [3.8-6.8]) and body control (1.5 [1-1.2] vs. 4 [2.8-5.8]) experienced.

Section 6.04 Discussion

This exploratory study investigated the effects of 8h HBF unloading and then 4h reloading with the Mk VI SkinSuit on parameters of elongation. The main findings were that 8h HBF unloading resulted in significant stature elongation, coupled with an increase in the anterior height of three cervical IVDs and one lumbar IVD. This is the first time that increases in anterior IVD height have been observed with unloading using the NASA ultrasound protocol. Reloading with the SkinSuit reduced stature significantly by 1cm upon donning. With a further 4h HBF unloading, this stature was maintained. Significant effects of SkinSuit reloading upon cervical or lumbar anterior disc height were not observed, although a trend for an increase in two lumbar discs and a decrease in another was seen. No SkinSuit integration issues were reported, though as before (Chapter 4) the SkinSuit increased movement discomfort and body control.

Effects of unloading and reloading on stature

Stature elongation experienced in the present study following 8h HBF of 2.1cm were near identical to that reported in previous 8h HBF studies in Chapter 4 during the day of 2.1cm and Chapter 5 overnight of 2.1cm. These consistent results may indicate that at 8h an initial plateau is reached with this analogue, which may increase with further unloading. Further studies using longer protocols are recommended as similar observations were made in a 3-day bedrest study which resulted in greater elongation at the end of the study compared to the first day (Styf *et al.*, 1997). Similar two-stage elongation was also reported from spaceflight (Thorton and Moore, 1987). This present study was the first in which participants were reloaded with the SkinSuit as opposed to comparing a loaded vs unloading condition. Reloading is more analogous to the operational situation in space. In this study, stature at the end of 8h unloaded HBF compared to at the end of 4h reloaded HBF was 0.7cm lower. This indicates that despite the maintenance of the unloading axis on the HBF, the partial axial loading imparted by the SkinSuit is resisting further unloading. However, it is also possible that 4h reloading is potentially not long enough to effect the IVD's, requiring further imaging studies using this testing protocol.

Ultrasound measurement of anterior IVD height

Ultrasound was chosen as the imaging modality for imaging the IVD spaces due to its portability and previous implementation on Earth (Ledsome et al. 1996) and also on the ISS (Marshburn *et al.*, 2014). The protocol used in space was successfully replicated in the present study with both the cervical and lumbar IVD visible. Imaging of the cervical discs showed a significant increase following 8h HBF, which combined was 2.3mm corresponding to just over 10% of the average stature elongation encountered in the present study after 8h HBF. A study on diurnal elongation attributed this to areas of the spine indicating 40% of elongation was attributed to changes in the thoracic, another 40% the lumbar and the last 20% miscellaneous (Wing *et al.*, 1992). Results from the present study indicate that cervical disc swelling could have contributed to 10% of total elongation with the lumbar swelling of 2.9mm accounting for nearly 15%, less than would be expected. This is the first time an expansion of the cervical discs has been observed after 8h sleep (unloading). Further research with prolonged unloading of several days should investigate if there exists a time course to this IVD swelling, which could provide information on the mechanisms behind the increased risk of herniation in the cervical spine (Belavy *et al.*, 2016).

Following 8h unloading the anterior height of three cervical IVDs (C3/C4, C6/C7, C7/T1) was significantly increased. Initial SkinSuit reloading did not result in any further significant differences (or trends) in cervical IVD height. At the end of the 4h SkinSuit reloading period, each IVD space had decreased marginally by between 0.2-0.4mm, albeit non-significantly. In the previous study (Chapter 4), which used MRI after 8h overnight HBF, there was a significant decrease in the posterior height of C7/T1 with SkinSuit loading compared to control. Whilst it is important to consider that unlike this current study, study participants had to be transported to imaging thus potentially confounding results, it may indicate the SkinSuit is exerting a tension on the cervical region. This tension may be arising from a pull on the cervical paraspinal muscles by the SkinSuit, thereby acting on the processes, resulting in compression. In microgravity, the muscle tension may be diminished more than on Earth due to the lack of resistance to the weight of the head to induce mechanical stress on the IVD, thus they may be more receptive to this imparted loading induced tension. This could be investigated further using a method to assess the stiffness (or elastic recoil using a Myoton device) of the supporting superficial neck muscles (Agyapong-Badu *et al.*, 2016), to determine if there is a relationship between muscle tension and disc height swelling in the cervical region, with/without loading imposition.

An important consideration with imaging is the technique employed as this can influence both the data acquired and the interpretation. In the bedrest study, a prone position was utilised to image the transverse processes to determine the change in the distance between the L1-L4 processes, which corresponded to 5.3mm after 8-hours of bedrest (Ledsome et al. 1996). Whilst in the NASA study a tethered supine position was used to image the anterior IVD spaces in space (Marshburn *et al.*, 2014a). As the present study was replicating the use of the NASA protocol and training tool, the results are not directly comparable with those acquired in the previous bedrest study, however the sum of the lumbar IVD height change observed anteriorly in the present study was 2.9mm, with the greatest change seen in the L5/S1 disc space, less than the bedrest study. Reasons for this discrepancy could be change in lumbar curvature, whilst a previous study looking at the effects of 8-hour bedrest on the lumbar spine indicated up to an 8mm elongation in the lumbosacral span with no change in lordosis, the imaging (using stereoscopic photography) was done when weight-bearing (Wing *et al.*, 1992), whilst in the present study imaging was done whilst maintaining a non-weight bearing position, which could be more sensitive to interactive changes in lumbar curvature. The limitations of ultrasound imaging as it does not capture the 'full picture' of what is happening to the lumbar spine. More detailed imaging using MRI is therefore recommended to further investigate these changes. The advantage of ultrasound is that it is portable and can be employed multiple times without disturbing the subject as demonstrated in the multiple time points collected in the present study over the 8h unloading + 4h reloading periods. As there are little data emanating on the time course of elongation and IVD swelling from longer term unloading, particularly pertaining to the cervical discs (Belavý *et al.*, 2013), it would be of pertinence to spaceflight missions to catalogue this in-flight with ultrasound to determine if there is a continued increase or stabilising of IVD swelling.

With initial donning of the SkinSuit a significant decrease in the lumbar IVD anterior height at L5/S1 and increases at L2/L3 and L4/L5 were observed with reloading, though the individual differences make clear generalisations difficult. A study in children comparing the effects of carrying backpacks less than or greater than 10% bodyweight found in the groups which wore heavier backpacks both the total length and lumbar length of the spine were significantly reduced by ~18mm and 9mm, respectively, with a further 7mm attenuation in thoracic length. Though the present study was not counter-balanced, nor did it control the level of loading imparted, it does support previous work from diurnal studies (Wing *et al.*, 1992) that the major contributors to changes in total spinal length are the lumbar and thoracic regions (Walicka-Cupryš *et al.*, 2015). A confounding factor could be that for the lumbar assessments, the participants had to doff the SkinSuit to halfway as the ultrasound signal could not penetrate the SkinSuit's fibres, despite attempts to soak the material fibres in a manner similar to that employed for abdominal imaging of horses (Barton, 2011). Thus, whilst lumbar imaging was taken place the reloading stimulus was briefly not imparted, as such this may have confounded the results. Whilst cervical assessment was not impeded by the SkinSuit, ultrasound may not provide a suitable platform to evaluate this countermeasure's effects of reloading upon the lumbar spine, thus a follow-up study using MRI is recommended. An additional limitation of the technique was that not all disc spaces in all participants were visible, either due to bowel gas scattering of the ultrasound or user error, which would not present an issue for MRI. Additional ultrasound studies could be run to determine if there is a more optimal approach to disc imaging to improve the signal quality.

Conclusion

Donning the SkinSuit in this exploratory study significantly reduced stature and prevented further elongation whilst on the HBF, with significant initial effects upon the anterior height of the lumbar spine. Ultrasound is readily adaptable and portable, however it is easier to image the cervical spine with ultrasound than the lumbar. This is in part due to reduced signal interference from stomach gasses and impracticalities of SkinSuit wear during lumbar imaging. Thus, due to these practical limitations it can only provide limited detail on the effects of reloading with the SkinSuit upon the lumbar IVDs, to facilitate countermeasure evaluation. Further ground studies using this protocol are required to elucidate whether SkinSuit reloading impacts the lumbar geometry and potentially the kinematics (Chapter 7).

Data from these studies will hopefully assist in the understanding of future mission results from Thomas Pesquet upcoming mission to the International Space Station, where further operational testing of the SkinSuit will take place (Figure 30).

Figure 30.Thomas Pesquet being fitted for his Mk VI SkinSuit prior to the PROXIMA mission. Image credit ESA.

Chapter 7. Exploring the effects of 4-hour partial axial reloading via the Mk VI SkinSuit upon lumbar geometry and kinematics after 8-hour hyper-buoyancy flotation

Section 7.01 Introduction

It is well established that prolonged periods of unloading on the spine both in microgravity (Chang *et al.*, 2016) and during bed rest analogues on Earth (Belavý, Armbrecht and Felsenberg, 2012) can lead to adaptive effects on the lumbar spine. These include atrophy of the paraspinal muscles (Hides *et al.*, 2016), increased muscular fat infiltration (Kalichman, Carmeli and Been, 2017) and altered protein content of the discs including decreased glycosaminoglycan (Jin *et al.*, 2013; Kordi *et al.*, 2015) and proteoglycan content (Yasuoka *et al.*, 2007; Chang *et al.*, 2014) in both human and animal models.

The IVDs' viscoelastic response is dependent on fluid flow responding to loading and unloading phases (Hendrik Schmidt *et al.*, 2016; Veliskova *et al.*, 2017). This cyclic loading imparts the required mechanical stimuli both for nutrient transport across the IVD (Huang, Urban and Luk, 2014) and cellular signalling with cartilage formation and regeneration responding to these signals (Mellor *et al.*, 2017). Whilst increased swelling has not been observed in space it can be inferred from increases in stature recorded in-flight (Thorton and Moore, 1987; Sudhakar *et al.*, 2015), which have been used on Earth as surrogate measures for changes in spinal height attributed to lumbar IVD swelling (McGill and Axler, 1996) and reductions in lumbar lordosis. Lumbar IVD swelling has been observed with both acute and long duration unloading analogues with 3-day dry immersion measuring +11±9% increases in disc volume at L5-S1 (Treffel *et al.*, 2016) and with 60-day bedrest increases of between 7.5-10.7% at L4/L5 (Kordi et al. 2015). Following unloading it can take up to two years for the IVDs to recover fully (Kordi *et al.*, 2015). Implementation of exercise countermeasures during both spaceflight and bed rest have been shown to preserve some of the trunk musculature i.e. transverse abdominus and internal oblique, however atrophy of the posterior paraspinal muscles, chiefly the multifidus still occurs (Belavý, Gast and Felsenberg, 2017).

These factors are likely associated with the 4-fold increase risk of disc herniation in astronauts (Johnston *et al.*, 2010), in particular the prolonged mechanical unloading and swelling of the IVD (Sibonga *et al.*, 2008) . On Earth there is an increased risk of herniation first thing in the morning when discs are fully hydrated (Adams, Dolan and Hutton, 1987). Similarly, the increased prevalence of disc herniation post-spaceflight is proposed to be associated with the swelling of the IVD, stretching the posterior annulus fibres resulting in susceptibility to posterior herniation (Belavy *et al.*, 2016).

A post-flight comparison of astronauts performing a flexion movement after returning from space, observed a 'stiffening of the spine' using a video x-ray (Chang *et al.*, 2014; Sayson *et al.*, 2015). Firstly, the term stiffening maybe an inappropriate term, as stiffness was not directly measured and refers to the rigidity of an object and its resistance to deformation. What the authors measured using this technique, termed 'quantitative fluoroscopy', was a decrease in intervertebral range of motion (IV-ROM), which is the range of intervertebral movement. Quantitative fluoroscopy dynamic assessment tracks the spine during motion quantifying how the vertebral bodies are moving relative to each other and has been performed both passively in a recumbent position to mitigate muscular contribution and during a standing, loaded, active state (Mellor, et al. 2014; Du Rose & Breen 2016). Muscular contraction is one of the largest actors upon the spine and IVDs (Adams, 2015), therefore in order to investigate the independent effects of unloading and reloading of the lumbar spine passive, non-weight bearing motion analysis might provide a more appropriate method that upright flexion, to explore these effects without added induced variation from motor control into the intervertebral kinematic assessment (Du Rose & Breen 2016). Studies investigating intervertebral motion have sought to quantify how well the discs are moving, in essence how restrained they are (Panjabi, 2003). The restraint of an IVD relates to the neutral zone (NZ), which is the area in-vitro where under loading, the spinal segment moves with minimal resistance (Panjabi, 1992). The size of the NZ changes depending on the loading imposed from the passive and active structures/inputs where in vitro this would be from compression of the disc (Smit *et al.*, 2011), but in vivo from added influences of the intervertebral ligaments and muscles, affecting the overall range of motion. The greater the restraint, the lower the range of motion and NZ and the less lax it is, which may provide an in-vivo marker of restraint. Laxity is the ratio of initial attainment rate of intervertebral motion compared to global trunk motion in the first 10^o of trunk movement (Mellor *et al.*, 2009). Measurement of laxity might offer greater insight in-vivo, into how the discs are responding to movement under differing loading conditions (Breen, Dupac and Osborne, 2015). However, whether this observed intervertebral restraint is directly correlated to disc swelling is not known at this time, though restraint would likely decrease with reduction in disc height and water content, as a result of a reduction in tension in the annulus fibrosis and intervertebral ligaments (Adams, Dolan and Hutton, 1987). What has been observed is in patients who identify as having chronic non-specific lower back pain, a higher proportional motion sharing inequality (MSI) during passive bending motion is recorded (Breen and Breen, 2017). How the motion is shared by the intervertebral segments as they move through motion provides an expression of the degree of intervertebral 'control' during motion by studying the variability of segmental motion (MSV) and the inequality of restraint among intervertebral segments (MSI). In separate studies (Mellor *et al.*, 2014) MSV was also shown to be significantly higher in chronic back pain patients. A reported symptom both in-flight and on return to Earth is lower back pain (Chang *et al.*, 2014), therefore characterisation of MSI and MSV in response to loading stimuli could provide further insight into kinematic consequences of unloading/reloading.

A further issue with imaging investigations post-flight is the time from landing on Earth to scanning. It can often take several days post-flight, enough for attenuation of the IVDs. In a backpack trial, even brief exposure to additional loading (15 minutes) resulted in significant impact upon the lumbar spine, increasing lordotic curvature and decreasing IVD height (Shymon et al. 2014). Thus, diminishing the time from unloading to screening and optimising the method of measurement is recommended for any countermeasure evaluation.

Previous SkinSuit studies have found a significant attenuation of stature compared to control conditions (Chapter 4; Carvil et al. 2016), with some evidence of compressive effect on the lumbar spine (decreased length, IVD height reduction; Chapter 5). However, the effects of this countermeasure for reloading the lumbar spine, specifically IVD geometry and restraint (i.e. laxity and IV-ROM) are not known. Also, these pilot studies (Chapters 4 & 5) compared an unloaded condition directly with the SkinSuit loaded condition, not reloading (Chapter 6), which would be the operational scenario of putting the SkinSuit on in space. In space, the cyclic loading signal is lost due to prolonged microgravity unloading resulting in disc swelling. Therefore, an investigation into how reloading the spine will affect both the IVD as well as how the lumbar spine responds to movement, could help to infer future countermeasure development and deployment.

Thus, refinement of imaging protocols and modality is required to undertake further evaluation into the effect of reloading the lumbar spine with the SkinSuit. The hypothesis is that 4-hour SkinSuit reloading, in healthy male subjects will attenuate the effects of unloading on spinal geometry and kinematics, measured through MRI and quantitative fluoroscopy respectively.

The aims of this pilot study were to: -

1) Explore how 4-hour reloading of the lumbar spine, via the Mk VI SkinSuit affects parameters of lumbar geometry, chiefly the size of the IVDs,
2) Determine if reloading acts to increase intervertebral motion by comparing parameters of intervertebral restraint between loading conditions with passive unloaded flexion and extension motion.

Specific objectives for the present study were to measure differences in the following variables, in the same participants, with and without 4h SkinSuit reloading after 8h overnight HBF exposure:-

- For lumbar geometry: Lumbar length, lordosis and IVD disc height were measured from L1-S1 from a sagittal MRI scan. The average disc cross sectional area and volume were measured on the three axial slices passing through the IVD from L2-S1
- For assessment of intervertebral restraint during passive recumbent flexion and extension: The laxity, maximal IV-ROM, maximal translation and minimum disc height values of all levels were pooled for paired comparison of the presence or absence of 4h SkinSuit reloading. Motion sharing variability (MSI) and inequality (MSV) were measured and compared across the segments from L2-S1.

Section 7.02 Methods

Experimental Approach

This pilot study was approved by the South West 3 Research Ethics Committee (REC Reference: 10/H0106/65) and conducted at the Anglo European Chiropractic college (Bournemouth, UK), consisting of two sessions 1 month apart using a specialist imaging centre (Anglo European Chiropractic College, Bournemouth). A new production line of Mk VI SkinSuits was commissioned from Dainese (Italy) using suggested improvements from Chapter 5 (i.e. non-metallic components). Additional funding for the present study was provided by both the European Space Agency and the Radiological Research Trust. The main outcomes measures for the present study were stature as performed throughout this thesis, lumbar geometry using MRI and lumbar kinematics using quantitative fluoroscopy. The repeatability of measurement (ICC), the standard error of measurement (SEM), range and minimal detectable change (MDC) of the measures of lumbar geometry from 20 IVDs (Table 9) are provided below with the inter-rater reliability (ITR) compared with a radiographer.

- ITR: Anterior IVD height: ICC = 0.910 (0.836-0.951)
- ITR: Posterior IVD height: ICC = 0.813 (0.673-0.896)
- ITR: Cobb angle: ICC = 0.993 (0.979-0.997)

Table 9. Intra-observer reliability, variation of measurement and minimal detectable change of MRI parameters by the author.

Parameter	ICC (95% CI)	Mean	SD	SEM	Range	MDC
Anterior IVD height (mm)	0.952 (0.884-0.981)	12.1	0.52	0.19	1.7	0.32
Posterior IVD height (mm)	0.933 (0.840-0.973)	5.8	0.22	0.05	0.79	0.16
Middle IVD height (mm)	0.983 (0.974-0.989)	11.4	0.53	0.03	1.1	0.10
Spine Length (mm)	0.998 (0997-0.999)	204	1.7	0.03	2.2	0.08
Cobb Angle ^{o}	0.980 (0.997-0.999)	40.4	0.25	0.05	0.59	0.12

For lumbar kinematic measurements the intra-observer reliability (ICC), standard error of measurement (SEM) and minimal detectable change (MDC) for these variables has been determined using a large clinical database and is as follows:-

- Laxity: ICC = 0.84 (0.49-0.96), SEM = 0.04, MDC = 0.11 (Du Rose and A. Breen, 2016)
- IV-RoM $_{MAX}$: ICC = 0.94 (0.80-0.99), SEM = 0.76^0 , MDC = 2.1^0 (Du Rose and A. Breen, 2016)
- Disc height: ICC = 0.531 (-0.138-0.808), SEM = 0.75 Eq. mm, MDC = 2.07 Eq. mm (Breen, 2011)
- Translation: ICC = 0.782 (0.589-0.884), SEM = 1.96 Eq. mm, MDC = 5.43 Eq. mm (Breen, 2011)

Participants

Eight male participants (28±5y; 1.77±0.05m; 73±5.3kg) gave written informed consent to partake in the study and additionally to have fluoroscopy performed on them, due to the low radiation dose imparted. They were screened for suitability via questionnaire by the MRI intendant and fluoroscopy operator prior to the study. Participants were asked to undertake normal activity on the day leading up to the study but abstain from vigorous exercise. Each was measured and fitted for a Mk VI SkinSuit that provided on average 0.19±0.03Gz axial loading at the foot (ForceShoes, Xsens, Netherlands). Each acted as their own control.

Protocol

Participants arrived at the centre in the evening and slept for 8h on the HBF in loose sleeping attire. Upon waking a small (15 minute) comfort break was given to all participants. Depending on which condition was being tested during this break participants either put on the Mk VI SkinSuit or remained in sleeping attire, before lying on the HBF for a further 4h. Participants were then transported supine from the HBF directly to the MR scanner using an MR compatible trolley prior to any measurement. Stature measurements were recorded before and after 8h overnight HBF and after the 15-minute break, using a commercially available stadiometer (SECA, UK). Subjective scales of movement comfort (Corlett and Bishop, 1976), body control (Cooper and Harper, 1969), and lower back pain (Appendix) were asked after the 4h reloading/unloading period prior to MRI (Figure 31).

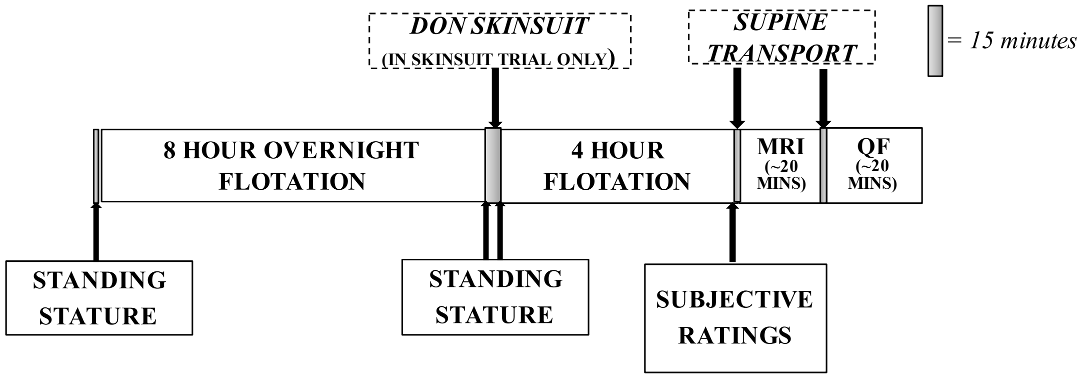


Figure 31. Schematic diagram detailing the 8h unloading and 4h reloading phases coupled with the taking of stature and subjective ratings before transport to MRI followed by quantitative fluoroscopy (QF).

Participants were positioned recumbent on their backs inside the scanner (Paramed MROpen 0.5T, Genoa, Italy) by the radiographer. Sagittal and axial scans were taken with eleven T2 weighted sagittal slices (5mm thickness, 2597/1117ms repetition/echo time, 30cm field of view), parallel to the spine on coronal localisers and 20 (four blocks of five slices) axial slices (4mm thickness, 5368/132ms repetition/echo time, 25cm field of view) aligned through each IVD L1-S1 to facilitate IVD height and cross-sectional area measurement (Figure 31).

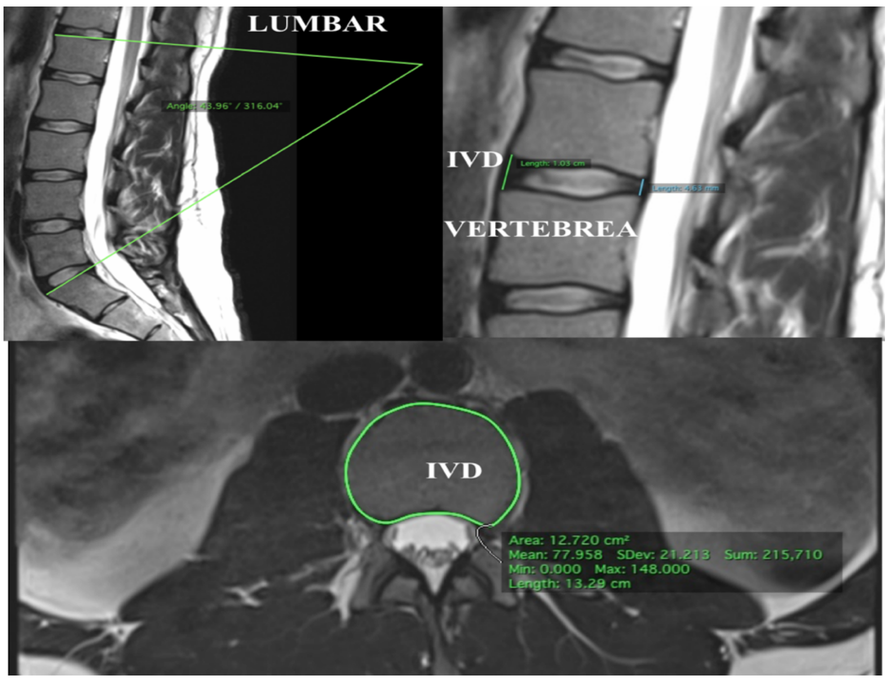


Figure 32. Image analysis of curvature (Cobb's angle), IVD anterior and posterior height (top) and IVD cross-sectional area (bottom). Image Credit King's College London.

After MRI participants were transferred supine to the x-ray room via trolley and poisoned on their left side, recumbent by a separate trained operator (Figure 33) upon a custom built motorised table driven by a controller (Atlas Clinical Ltd, Lichfield, UK).

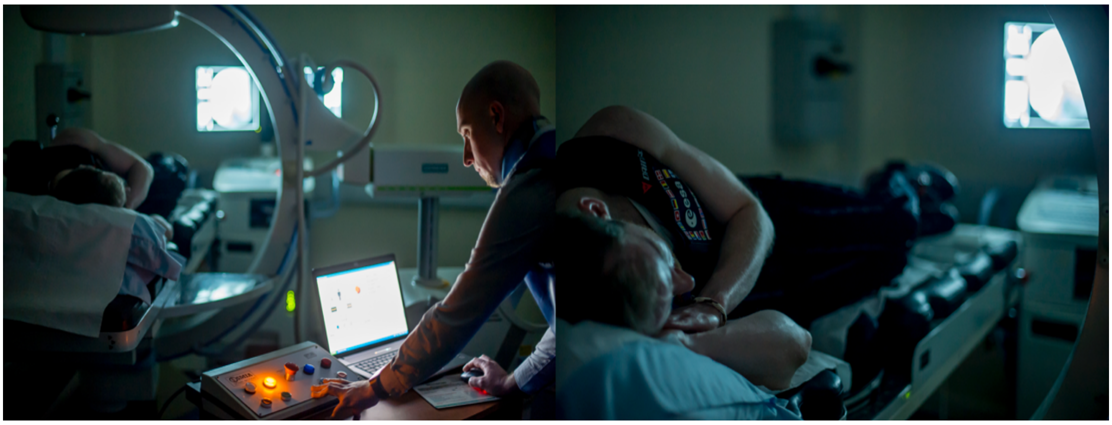


Figure 33. Participants were positioned recumbent, on their side on a motor controlled bed with the C-Arm fluoroscope positioned around the participant with the central array trained upon the L4 vertebrae (top). Image Credit AECC.

Prior to scanning participants were taken through the passive, recumbent, ranges of movement in stages, to ensure tolerance to flexion/extension angles and to standardise the position thereby reducing variability of measurement and influence of external factors (Breen *et al.*, 2012). Lead shielding was placed on the gonads, breasts and thyroid to minimise radiation exposure and to reduce flaring in the images. Fluoroscopic imaging was performed at 15Hz (Siemens Arcadis Avantic VC10A digital fluoroscope, Henkestrasse, Germany) and synchronised with the digital outputs from the motor of the motion frame. Participants were passively moved, by a computer controlled motor operated table, through 40^{o} flexion and 40^{o} extension movement over a period of about 20 seconds at 6^{o}s^{-2} for the first second of motion followed by 6^{o}s^{-1} (Breen and Breen, 2016). The central ray was positioned at L3-4 with all vertebrae from L2-S1 in view, with continuous imaging taken throughout this motion (Mellor et al. 2014). Image acquisition was repeated if there was an obstruction i.e. bowel gas in the view. Image processing and analysis was done using a custom-built script in MATLAB (V7.12, The Mathworks, Cambridge, UK), where each of the vertebral corners (L2-S1) are marked five separate times, processed to determine their movement during the dynamic sequence before a resultant average is calculated for each measured parameter (Figure 34).

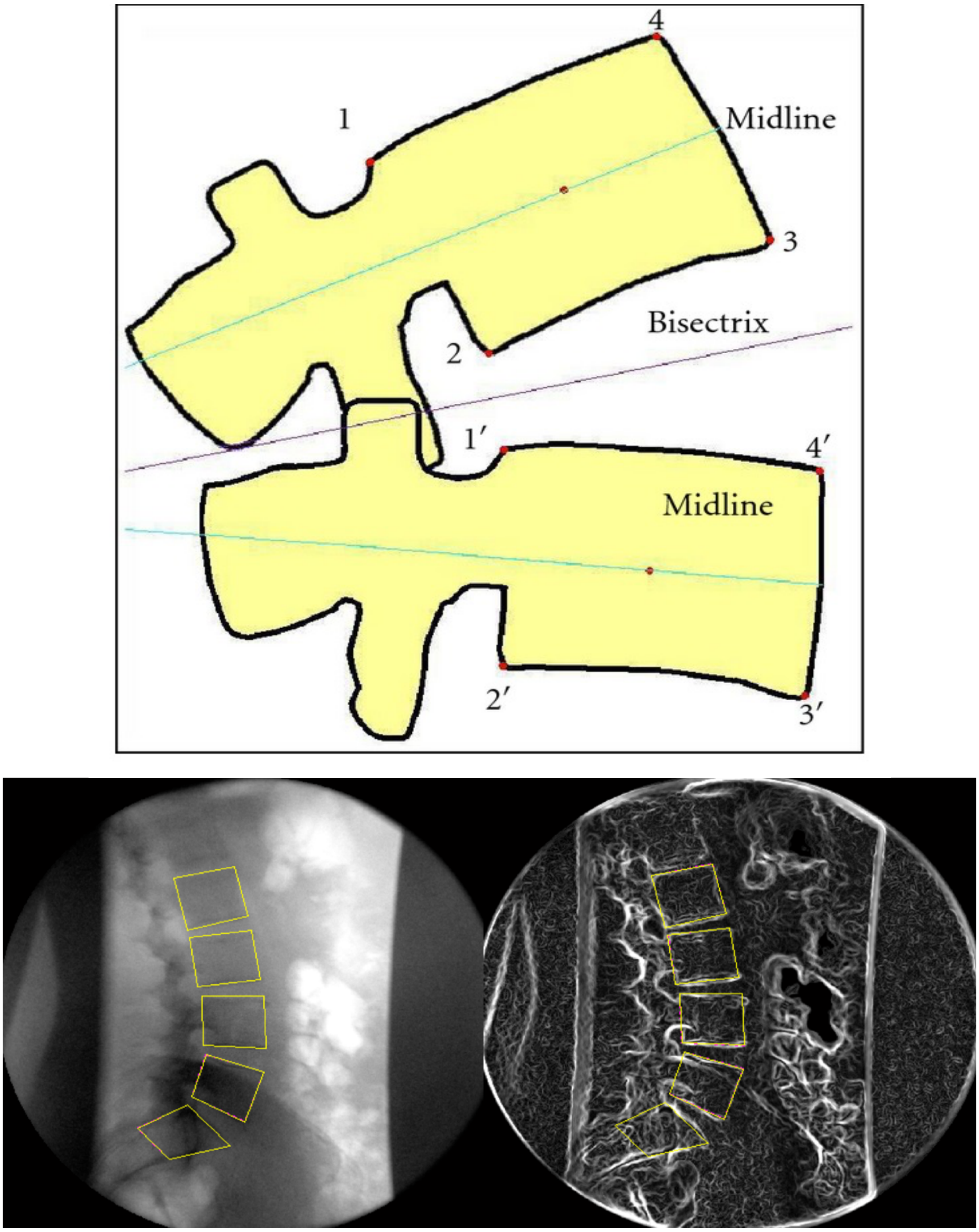


Figure 34. Top – Frobin's method for positioning of the four vertebral corner markers and the calculated midline of the vertebral segment and bisecting intervertebral level for velocity and angle measurement tracking (Frobin et al, 1996). Bottom - Raw fluoroscopic image of the lumbar spine (left) and a processed image (right). Templates are calculated by positioning four markers on the corners of each vertebrae that are tracked throughout the sequence. Image credit AECC, Bournemouth, UK.

The parameters measured at each intervertebral level were laxity, maximal intervertebral range of motion (IV-RoM $_{MAX}$), dynamic disc height and the maximal translation of the IVD observed during dynamic motion. Laxity is the initial attainment rate, that is the ratio between the slopes of the movement measured by the table (global motion) and the inter-vertebral motion (rotation) in the first 10^{o} of movement and is a refinement of the global attainment rate (velocity) of when IV-RoM_{MAX} is reached (Du Rose & Breen 2016). This provides an indication of the slackness of the IVD and has been correlated with the dynamic neutral zone of the disc thus providing an insight into the stability (Breen, Dupac and Osborne, 2015). IV-RoM_{MAX} refers to the maximal amount of angular change of position at the intervertebral level, recorded at any point in the moving sequence, providing the maximal range of motion and is measured through the angle produced through the midline of the vertebrae (Figure 34) (Du Rose & Breen 2016). The dynamic disc height is the smallest average disc height (calculated from the average of the anterior and posterior disc heights) recorded during the moving sequence, whilst the translation is the movement behaviour of the adjacent vertebrae in relation to the IVD from their respective central positions using Frobin's method (Breen and Breen, 2016), both of which are converted from VBU units to equivalent mm by multiplying by 35 (Frobin *et al.*, 1996).

Data analysis

All data was anonymised with the author blinded through random number assignment to scans prior to analysis. Images were checked by a consultant for any underlying pathology. Normality was assessed by visual check of histograms and whether the skewness and kurtosis ratio lay below or above 1.96/-1.96 (Fallowfield, Hale and Wilkinson, 2005). Data were compared between SkinSuit/non-SkinSuit exposure and expressed as either means ± SD (stature and MRI measurements – t-test) or median ± interquartile range (subjective ratings and QF measurements – Wilcoxon test).

Recumbent MR images were analysed using RadiAnt Dicom Viewer V1.19 (Medixant, Poznan, Poland). Lumbar spinal length was determined using the distance between the posterior superior corner of the L1 and S1 endplate. Cobb's method evaluated lumbar curvature through the angle formed between tangent lines drawn from the L1 and S1 superior endplates. Anterior, middle, posterior and average IVD height was determined using a modified Dabb's method - averaging the distance between the anterior, middle and posterior IVD from L1/L2 to L5/S1 (Chang *et al.*, 2016). IVD volume was calculated by multiplying the average height as measured above, with the average of the cross-sectional area taken by drawing the

IVD area from the three axial slices through the midline of the IVD (OsiriX Lite, Pixmeo Sarl, Switzerland).

Recumbent, side-lying fluoroscopic images and table outputs were transferred to Matlab (V7.12, The Mathworks, Cambridge) where a bespoke program tracked, frame to frame the vertebral body images after marking the L2-S1 vertebrae five separate times on the initial image before movement. Tracking was visually checked to ensure it stayed on the vertebrae during movement as obscuring of vertebral bodies can disrupt tracking e.g. from excessive bowel gas. Once confirmed an average of all five tracking's was taken and the single level outputs (L2/L3, L3/L4, L4/L5, L5/S1) for Laxity, IV-ROM$_{MAX,}$ translation$_{MAX,}$ dynamic minimum disc height (Figure 35) were pooled for a paired comparison of L2-S1 pooled changes between loading conditions. Motion sharing variability and inequality which are multilevel variables (L2-S1) were also calculated using the Matlab script prior to comparison (Breen and Breen, 2017). Statistics were performed using Statistical Package for Social Sciences 24.0 (SPSS IBM, Chicago, IL, USA). A previous spaceflight study using follow-up changes from spaceflight with MRI and QF (Sayson *et al.*, 2015) assumed a significantly powered result when p<0.20, based on a sample size of 12, so this investigation should be viewed as a pilot study due to its lower n number (n=8). Post-hoc sample size calculations were run using G*Power (Heinrich Heine, University of Düsseldorf, Germany) (Faul *et al.*, 2007).

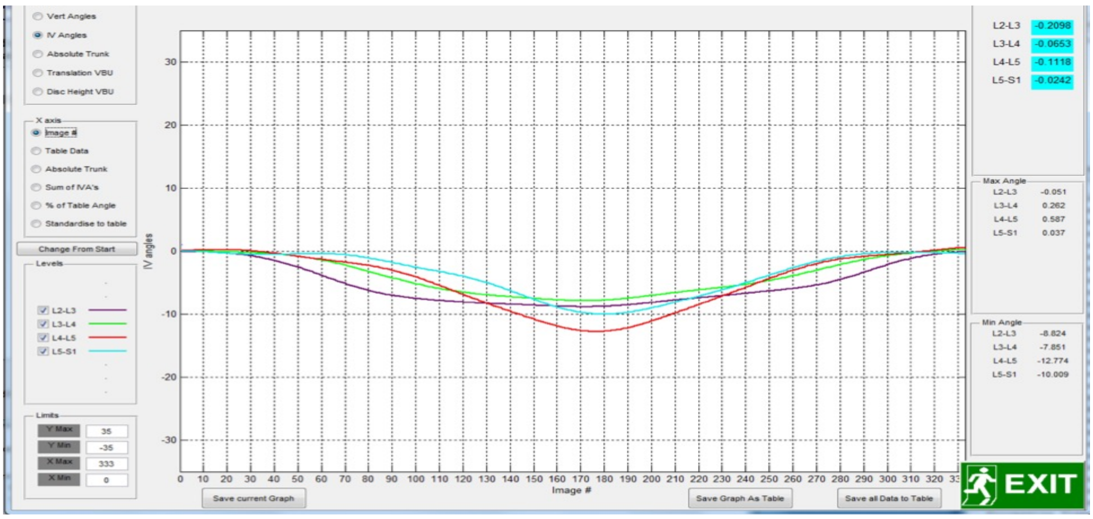


Figure 35. Example of the control GUI outputs for the averaged tracking of the four vertebral segments between L2-S1 (four lines) during movement for intervertebral angle motion, where the x-axis is image frame over time and the y-axis is the IV-angle used to determine IV-ROM. Image credit AECC, Bournemouth, UK.

Section 7.03 Results

All participants comfortably slept on the HBF overnight and could don the SkinSuit without issue. Stature was significantly ($p<0.0001$) increased after overnight sleep on both sessions (21±3.8mm). Preceding the 15-minute break and before donning the SkinSuit, stature had reduced by 6.1±2.5mm ($p<0.0001$), a 30% drop in stature gain. This gain was further reduced upon donning the SkinSuit by 4.5±6.5mm ($p=0.07$), which in total resulted in a 50% drop in stature gain at the start of SkinSuit reloading period vs. 30% without.

The clinical review of MR images reported incidental findings in three participants, each with one disc showing signs of degeneration either at L4/L5 or L5/S1. No follow-up was required. Comparing the unloaded and SkinSuit reloaded recumbent MRI images, neither lumbar length (138.8±6.4 vs. 138.9±6.8mm) or lumbar lordotic curvature (42±6.8 vs. 41.1±7.2^{o}) were affected by SkinSuit reloading. There was a trend ($p<0.2$) for a reduction in average IVD height measured at L3/L4 (0.44mm) and L4/5 (0.34mm). No other significant differences were observed (Figure 36).

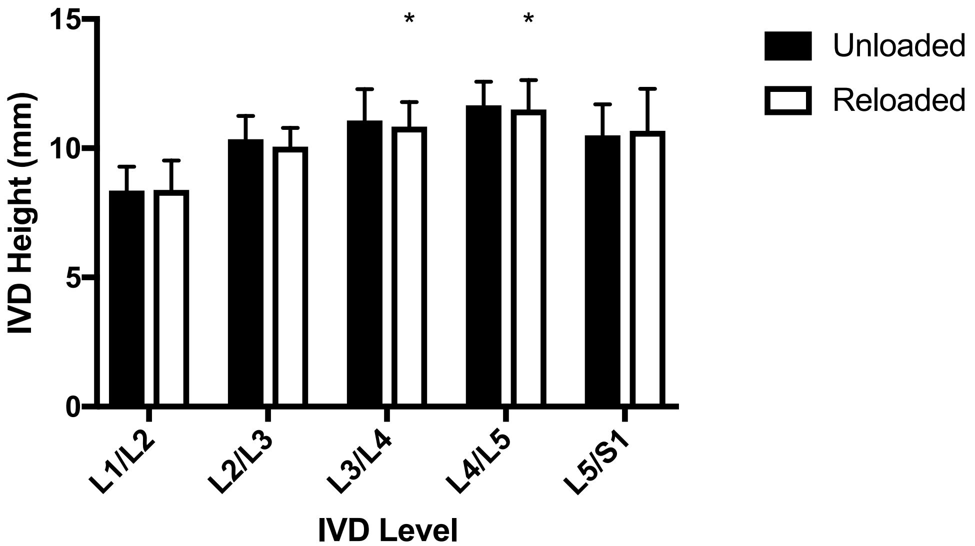


Figure 36. IVD height (mean±SD) between the two loading conditions. * Trend ($p<0.2$) between loading conditions.

There was a further trend for an increase in IVD cross sectional area with reloading at L4/L5 ($p=0.07$), L5/S1 ($p=0.09$) and a decrease in the volume at L2/L3 with recumbent MRI ($p=0.05$) and L4/L5 ($p=0.12$; Table 10).

Table 10. IVD cross sectional area and volume (mean±SD) compared between loading conditions when recumbent.

IVD Level	IVD Cross Sectional Area mm^2		IVD Volume mm^3	
	Unloaded	Reloaded	Unloaded	Reloaded
L2/L3	1611±232	1603±222	8871±2846	*8339±2299**
L3/L4	1638±236	1639±181	9806±3167	9400±2234
L4/L5	1716±295	*1770±287*$^{\$}$	11343±4003	11907±4470$^{\$}$
L5/S1	1490±204	*1531±230*$^{\$}$	7727±2534	8500±3945

$^{\$}$ Trend (p<0.2) observed between loading conditions. ** p<0.05*

During flexion, all parameters possessed a marginally higher median with reloading apart from disc height with was lower, there were significant differences in several parameters (Table 11).

Table 11. Parameters of intervertebral restraint (median±IQR) compared between loading conditions during 40^{o} recumbent passive flexion (from neutral).

Parameter L2-S1	Condition		
	Unloaded	Reloaded	*p-value*
Laxity	0.13 (0.10-0.18)	0.14 (0.09-0.19)	p=0.69
IV-ROM $_{Max}$ ^{o}	4.2 (3.17-5.71)	4.94 (3.73-6.49)	*p=0.03**
Translation $_{Max\ eqmm}$	0.08 (0.08-0.12)	0.1 (0.07-0.11)	*p=0.03**
Disc Height $_{Min\ eqmm}$	0.26 (0.22-0.44)	0.24 (0.2-0.3)	*p=0.01**
Motion Sharing Variability	0.04 (0.03-0.05)	0.04 (0.03-0.05)	p=0.88
Motion Sharing Inequality	0.21 (0.17-0.24)	0.26 (0.19-0.31)	p=0.67

During extension, there was an observable increase in several of the parameters with reloading with significance for MSI (Table 12). Though marginal reductions in laxity and IV-RoM_{max} were observed these were also not significant.

Table 12. Parameters of intervertebral restraint (median±IQR) compared between loading conditions during 40^{o} recumbent passive extension (from neutral).

Parameter L2-S1	Condition		
	Unloaded	Reloaded	p-value
Laxity	0.14 (0.06-0.18)	0.11 (0.08-0.14)	*p=0.23*
IV-ROM $_{Max}$ ^{o}	5.04 (4.2-06.63	3.92 (3.08-5.9)	p=0.42
Translation $_{Max\ eqmm}$	0.10 (0.06-0.13)	0.11 (0.8-0.13)	p=0.35
Disc Height $_{Min\ eqmm}$	0.29 (0.23- 0.45)	0.28 (0.23-0.35)	p=0.35
Motion Sharing Variability	0.05 (0.03-0.06)	0.05 (0.03-0.09)	p=0.67
Motion Sharing Inequality	0.24 (0.22-0.26)	0.34 (0.29-0.37)	*p=0.12*

During the reloading phase, wearing the SkinSuit (compared with sleeping attire) led to a small but significant increase in the degree of movement discomfort (2 [2-2] vs. 4 [4-4.3], $p<0.008$) and body control (1 [1-1.5] vs. 3 [3-3.3], $p<0.011$) experienced. No reports of back pain were communicated whilst wearing the SkinSuit though 2 individuals reported 'stiffness' proceeding the 4h morning HBF phase without the SkinSuit.

Section 7.04 Discussion

All participants successfully completed both conditions without incident, with the SkinSuit imparting 0.19±0.03Gz during the reloading condition at a tolerable comfort level. The hypothesis that reloading after enhanced unloading would act to reduce disc height and decrease measures of intervertebral restraint is reasonable. No significant difference in lumbar length or curvature were observed. However, a tendency with reloading for a reduction in the average IVD height at L3/L4 and L4/L5 was seen. IVD cross-sectional area increased with SkinSuit reloading at the lower lumbar levels (L4/L5, L5/S1). with a trend for a decreased volume at L2/L3 specifically. Reloading during recumbent spinal flexion resulted in significant, minor increases in several measures of intervertebral restraint and a decrease in disc height. During recumbent spinal extension, nonsignificant reductions in range of motion and laxity (or increased restraint) were observed with reloading with an increase in MSI.

Immediate effects of SkinSuit reloading

Eight hour HBF unloading resulted in significant stature elongation, in line with previous HBF studies (Carvil et al. 2015; Carvil et al. 2016). Upon rising, after 15 minutes of weight bearing a 30% reduction in stature elongation (6.2mm) was recorded. This reduction is within the boundaries of that reported in literature, with 54% lost within the first hour of rising (Tyrrell, Reilly and Troup, 1985). Donning the SkinSuit at the start of the 4h reloading, lowered incurred stature elongation further by 20% (4.5mm). A study using 15% bodyweight backpack loading found similar degrees of stature reduction after 10 minutes with both front and back loading (Chow *et al.*, 2011). Whilst the time of day is not reported in their study, the authors did use a linear variable differential transformer to measure height. This is a preferred method of stature measurement (Tyrrell, Reilly and Troup, 1985) as it can more readily mitigate confounding influences, such as postural influences. The SkinSuit imparted on average 0.19Gz, more than the 15% used in the backpack trial and previous SkinSuit trials (Chapter 4, 5, 6) . This could be due to improvements in SkinSuit sizing, as it is calculated by design to impart 0.2Gz, however improper fit can reduce Gz loading (Kendrick, 2016). This increased loading and axial direction might explain the increased rate of compression compared to backpacks. In total, prior to the 4h reloading period participants has lost nearly 50% of the elongation induced by HBF, as opposed to 30% in the control condition.

<u>Effects of reloading on lumbar length and lordosis</u>

After the 4h reloading period, supine MRI did not observe a significant difference in lumbar length or curvature with SkinSuit reloading applied. A study investigating differences between supine, upright and upright + a 10% bodyweight backpack also did not find changes in length or curvature, despite the increased loading on the spine (Shymon et al. 2014). The duration of loading was far less in that study (>8 minutes) than this one (> 4 hours). Scanning was also performed upright whereas loading in the present study was applied in a supine state. SkinSuit reloading did show a tendency to decrease the average height of the IVDs specifically at L2/L3 and L3/L4 by 0.24-0.28mm.

Previous studies have used reloading via a harness in supine scanning to mimic upright conditions (Lee *et al.*, 2003). One study which used 11 minutes of 48% bodyweight reloading found a significant reduction in L4/L5 height of 0.8mm (Kimura *et al.*, 2000). The authors also note a reduction in lumbar length of 2.5mm which the present study did not find. These discrepancies despite the increased reloading period in the present study could be due partly to the increased load, but also the manner to which it is transmitted down the spine. When loads are imparted vertically the loading capacity and resistance to buckling is reduced, whereas when the loading follows the curve of the spine the load capacity increases, this is known as a follower load (Patwardhan *et al.*, 1999). A study on displacement of lumbar spine ex-vivo found with vertical loading the spine buckled under 100N of load, whereas it could take over 1000N if following the spine (Patwardhan *et al.*, 1999).

The SkinSuit works axially, in-line with the body, imparting approximately 20% incremental loading at the foot through multiple 4cm stages. Load – force relationships of this staged loading have shown that as more of the suit is stretched, the force produced increases in a near linear fashion (Stoppa, 2016). This is similar to the linear spring relationship described in Hooke's law which has been utilised in exoskeleton designs (Zhang, 2014). This would result in 50% of the SkinSuits loading (~0.10Gz) being imparted at the mid-point of the SkinSuit i.e. the hip at

L4/L5, thus changes in curvature may not be expected. However it is noted that alterations in the friction interface of the SkinSuit with the person i.e. slippage can affect the length-force relationship (Kendrick, 2016; Stoppa, 2016). Previous SkinSuit studies also did not show a significant effect of loading on curvature, though in that study 4/6 participants reported increased curvature with loading. This conflicting finding may be in part due to differences in study protocol. SkinSuit loading was applied for 8h at the end of the day as opposed to the start of day in this study. At the end of the day when the spine has been loaded tissues are more elastic and enjoy a greater range of motion (Adams *et al.*, 1990). Thus, as reloading was applied during the morning on an unloaded spine in this study, the differences between studies is not unexpected, and more closely resembles the nature of donning the SkinSuit in space.

Effects of reloading on intervertebral disc geometry and volume

SkinSuit loading also marginally increased the cross-sectional area at the lower lumbar levels (L4\L5, L5/S1). A study compared the effect of 20 minutes of walking with a 20% bodyweight vest, on IVD geometry (Lewis and Fowler, 2009). A pooled decrease (L1-S1) of 0.9mm^2 of IVD height was recorded with a 35mm^2 increase in cross sectional area with loading, whereas the pooled decrease in IVD height was 0.5mm^2 for the present study with an increase of 30mm^2 in cross sectional area. The upright walking plus the vest resulted in slightly greater IVD deformation potentially due to increased loading of the disc applied by the vest and potential concurrent effects of exercise (Kingsley *et al.*, 2012). Overall, a tendency for a decrease in the overall volume of L2/L3 and L4/L5 with reloading was observed. However, this value was calculated not measured directly, whereas other studies have used 3D volume analysis (Botsford, Esses and Ogilvie-Harris, 1994; Treffel *et al.*, 2016). Therefore, whilst these results should be treated with caution they do suggest that reloading with the SkinSuit is applying a compressive load through the spine during static assessment.

Spinal kinematic measurement

Quantitative fluoroscopy has been employed to evaluate the effect of spaceflight on active spinal flexion (Chang *et al.*, 2014). In a group of five astronauts it was reported that the extended unloading from space resulted in an increased 'stiffening' of the spine (Sayson *et al.*, 2015). The authors base this on a reduction in IV-ROM. However, a change in IV-ROM does not directly measure disc stiffness, but bending stiffness (O'Connell *et al.*, 2011). It suggests that the prolonged unloading on the spine acts to inhibit intervertebral range of movement. This may in turn indicate an increase in the level of intervertebral restraint.

IV-RoM_{MAX}, Laxity and Translation are indicators of single level restraint, whereas MSI is a measure of the equality of multilevel restraint and MSV is a measure of its variability (Breen & Breen 2017; Breen et al. 2015). Disc height is a measure of disc compression (Schmidt et al. 2016), which is not itself a marker of segmental instability (Hake *et al.*, 2002). Sustained compressive loading over time decreases disc height and water content, therefore reducing markers of restraint. However, acute compressive pre-loading of the disc, increases nucleus pressures and stiffens the annulus fibres of disc (Schmidt et al. 2016). Prolonged unloading increasing disc height results in an increased strain on the annulus fibres decreasing their elastic limit, and thus decreasing ROM (Laws *et al.*, 2016). In an in-vitro study of human IVD subjected to axial loading, it was observed that during the unloaded phase, as disc height recovered, so too did the stiffness (O'Connell *et al.*, 2011). The recovery time of the disc was also considerably longer than the loading. Thus, increased swelling of the IVD can lead to increased resistance to forward bending, decreasing the range of motion and increasing the restraint, where applying sustained compressive loading opposes this. This notion tends to be supported in this study.

Effects of SkinSuit reloading on passive recumbent flexion and extension

With sustained creep loading the range of motion during flexion increases due to decreased disc height and water content (Adams, Dolan and Hutton, 1987), however during extension this effect is balanced by increased resistance of the spinous processes and apophyseal joints (Adams *et al.*, 1990). In the present experiments, participants were measured dynamically, in passive recumbent motion, after enhanced unloading. The studies in NASA astronauts found enhanced unloading from spaceflight to be possibly associated with increased restraint (Sayson *et al.*, 2015). However, the techniques they used were not described in detail and they also employed a standing protocol which would add variable factors into the results from mechanical loading, motor control and muscle tone. Reloading with the SkinSuit during flexion led to a minor, non-significant increase in the laxity and IV-ROM during flexion, with an increase in these parameters during extension. Loading of the disc during flexion could be working in two ways, first to reduce the height of the disc, thereby reducing the annular strain, but also by compressing it increasing the annular stiffness. If reloading had the effect of reducing restraint due to reduction in peripheral annular tension, laxity, IV-RoMmax, and translation should be greater after reloading. Annular tension may be considered to have a damping effect on any differences between levels in terms of their restraint and the variability thereof. Therefore, reduced annular tension from reloading would increase MSI and MSV. This apparent increase was observed in both flexion and extension in the study. However, during extension, both laxity and IV-ROM were reduced meaning that even though measures of restraint were increased with reloading, increasing bending stiffness, the variability and inequality also increased suggesting there are potentially other influences on motion control i.e. muscle or ligament tension.

A study of lumbar disc pressure measurements in different postures deduced that the average disc pressure for a 70kg man at L3 was 200N when lying down, 500 standing normally and 1000N when bending at 40^{O} flexion (Nachemson, 1981). In extension, intradiscal pressure reduces as the compressive force is resisted by the spinous processes, apophyseal joints and to an extent the posterior ligaments (Adams *et al.*, 1994). With sustained creep loading decreasing disc height and bending stiffness, resistance sharing is increased (Adams *et al.*, 1996). This decrease in disc height would be expected to result in greater elasticity and range of motion. Therefore, the paradoxical finding in the present study of decreased IV-ROM and laxity with reloading, despite decreases in disc height with continued loading is intriguing and might be related to induced muscular influences imparted acute preloading on the disc, by resisting the SkinSuit in this recumbent posture. Computer simulations on the effect of SkinSuit loading in weightlessness have demonstrated increased muscle activity in response to overcoming the resistance imparted by the elastic material of the SkinSuit (Kendrick and Newman, 2014). Whilst speculatively this may be a contributing factor to the results, as interacting structures can affect the disc loading response. For example removing both ligaments and the apophyseal joints in-vitro has been seen to affect how the disc behaves during extension (Adams *et al.*, 1996).

Compressive forces on the spine have been found to increase trunk muscle activity, particularly the muscles of the upper erector spinae group which run parallel to the compressive axis (Callaghan and McGill, 1995). With a 15% bodyweight backpack (0.15Gz) positioned posteriorly there was an increase in rectus abdominus and a decrease in erector spinae activity, whereas with front loading erector spinae activity increased (Motmans, Tomlow and Vissers, 2006). Whilst muscle activity should be silent in a passive recumbent state, the requirement to overcome resistance imparted by the SkinSuit could be inducing increased extensor activation in this passive, unloaded state. Application of push-pull springs applied to the lower spine posteriorly in exoskeleton development have demonstrated an effect upon muscle activity and reducing intervertebral torque (Zhang, 2014). Thus, as no measures of spinal muscle activity were taken, despite the recumbent position potential muscular influences from overcoming the elastic element of the SkinSuit cannot be discounted. An increased extensor activity would act to increase the resistance to bending, as the extensor muscles run parallel to its compression axis (Adams and Hutton, 1986). Increased activation of extensor muscles might therefore act to increase the restraint on the disc (laxity, IV-ROM) but also the disc compression and variability of motion sharing (Breen and Breen, 2017). These interactions, while purely speculative, are nonetheless consistent with the tendencies observed in the data. Therefore, future studies should consider the measurement of muscle activity to characterise whether the acute and prolonged compressive axial loading of the SkinSuit effects trunk muscle activity. Comparison with weight-bearing flexion and extension as performed in the NASA study with the addition of SkinSuit loading should also be investigated as this would include paraspinal muscle influences in intervertebral motion (Du Rose & Breen 2016).

Future directions

Based on these findings future studies investigating the effect of load and unloading on kinematics should seek to use measurements of laxity, MSI, MSV and potentially disc height and IV-ROM$_{MAX}$ as translation does not appear to be sensitive enough to determine a suitable effect. Similarly, measurement of lumbar lordosis when recumbent is variable, possible due to variation in participant position during scanning, therefore this should seek to be standardised further before measurement i.e. with upright positioning in future assessments. Three-day unloading has been performed using spaceflight analogues including dry immersion and has reported significant increases in disc swelling and water content using MR spectroscopy (Treffel *et al.*, 2016). Future studies should seek to optimise both the duration of HBF and the measurement of lumbar structure and kinematics to determine the effect of prolonged HBF on spinal geometry and the relationship between disc swelling and kinematics.

An opportunity for future research could be to quantify the kinematic effects of load/unloading upon the cervical spine. In Chapter 6, increases in cervical anterior disc height were observed with unloading. An investigation into the effect of cervical spinal manipulation, found a dose-relationship between manipulations and IV-ROM suggesting a mechanical influence on vertebral segments (Branney and Breen, 2014). As this is also an identified high-risk area for herniation in astronauts (Johnston *et al.*, 2010), further study into the relationship (if any) between disc swelling on cervical kinematics could aid in the understanding of the mechanisms for cervical herniation.

Finally, in the NASA study the authors concluded that in order to have a suitability powered study to detect post-space flight changes in lumbar kinematics and geometry a sample size of 12 is required (Sayson *et al.*, 2015). Though there are constraints of astronaut recruitment which places limitations on sample sizes, these authors do not explain which measures they used to calculate this sample size. They also suggest due to the large variability measures including lumbar geometry have low effect sizes and do not change but this more likely due to the time taken getting astronauts to the scanner post spaceflight. The present pilot study did observe minor changes in both kinematics and geometry despite its low sample size (n=8).

Based on these observations future studies into reloading could look to improve sample sizes to arrive at a suitability powered study. The number of participants calculated for variables in the present study for suitable power (β=0.8, α=0.05) are: -

- QF-Laxity: 52
- QF-MSV: 46
- QF-MSI: 33
- QF-IV-ROM$_{MAX}$: 2546
- QF-Disc height$_{min}$: 241

- QF-Translation: 6415
- MRI-Lumbar lordosis: 100
- MRI-IVD CSA: 17
- MRI-IVD volume: 34

Figure 37. Thomas Pesquet wearing the Mk VI SkinSuit aboard the ISS whilst conducting other experiments. Image credit ESA/NASA.

Conclusion

In this study, it was hypothesised that reloading with the Mk VI SkinSuit, after 8h HBF unloading, would reduce disc height and measures of intervertebral restraint through compression. The minor reductions in disc height, volume and increase in cross sectional area with SkinSuit reloading, coupled with indications of attenuated restraint during flexion, suggests that this may be occurring. Paradoxical findings of minor increases in measures of intervertebral restraint during extension, suggest that in addition to mechanical reloading, additional factors such as muscle activation may be influencing motion control.

It has been suggested that due to the increased swelling of the discs in space, this could lead to increased fibre strain, reducing the elastic limits of the annulus fibres. However only intervertebral range of motion was measured in the NASA study during flexion. In order to better understand the effects of prolonged unloading on the IVDs and the precipitation of increased risk of hernias, incorporation of parameters used in the present study i.e. laxity, MSV and MSI should be considered.

Replication of these studies with consideration for appropriate sample sizes and incorporation of motor control measurement would also provide further insight into the effects of unloading the potential utility of the SkinSuit to reload and support the lumbar spine in space.

Chapter 8. General discussion

This thesis sought to evaluate the European Space Agency's Mk VI SkinSuit, a proposed countermeasure for microgravity-induced spinal elongation, by exploring its effects upon parameters associated with unloading of the spine. An appropriate analogue platform that would induce unloading and thus spinal elongation was first tested, to facilitate countermeasure evaluation as previous spaceflight analogues are considered unsuitable for spinal evaluations and/or inaccessible. A pilot study initially explored the effect of Mk VI SkinSuit loading upon spinal elongation using stature as a surrogate measure of spinal elongation. This was accompanied with imaging techniques to observe load/unloading effects upon the lumbar intervertebral discs, that were compatible with the SkinSuits metallic components (DEXA). Design modifications were recommended and actioned to facilitate wider evaluations on the effects of loading and unloading using gold standard imaging modality (MRI). With the SkinSuit modified and piloted with MRI to ensure compatibility, the study design was optimised to better parallel the operational scenario of donning the SkinSuit in space - that of reloading an unloaded spine. An exploratory assessment was undertaken in the first instance to pilot this new study design using a NASA ultrasound protocol. This provided unique insights on this mode of assessment for cervical IVD unloading. This reloading study design was implemented into the final experiment, which compared the effects of SkinSuit reloading vs. unloaded on geometric and kinematic parameters of the lumbar spine.

This thesis provides the first pilot data and contribution to knowledge on: -

1) A novel microgravity-unloading analogue; hyper-buoyancy flotation
2) ESA's Mk VI SkinSuit and the effects of its imparted axial loading upon stature elongation, spinal length and IVD height
3) Effect of reloading via the SkinSuit upon lumbar IVD geometry and kinematics

Section 8.01 Hyper-buoyancy flotation

Currently there are several methods of inducing microgravity-like unloading conditions for human studies on Earth. Analogues including parabolic flight, head up wet immersion and suited immersion offer short windows (~22s to a few hours) of microgravity-like conditions before termination is needed (Barr, Clement and Norsk, 2015). These methods offer sufficient time to allow operational evaluations and the study of rapid responses to microgravity. However, platforms that offer the facility for longer-term evaluations are required both for the study of physiological adaptations and countermeasure development. Dry immersion and head-down tilt both have facilitated long term investigations ranging from a few hours to several months (Navasiolava et al. 2011; Belavý et al. 2010). However, limitations in both platforms suggest they may not be ideal for the evaluation of spinal countermeasures. Dry immersion has recently been shown to induce swelling of the lumbar IVDs after a few days (Treffel *et al.*, 2016). However immersion of the subject in water means they are less accessible, compression forces are imparted by the water and there is an axial vector on the head (Navasiolava et al. 2011; Andrade et al. 2014), which combined is not optimal for spinal countermeasure evaluation. Head down tilt has also been shown to induce lumbar IVD swelling (Belavý et al. 2012), paraspinal muscle atrophy (Belavý et al. 2017) and significant stature elongation (Styf et al. 1997). However its titled position means hydrostatic gradients are not representative of space (Hargens and Vico, 2016). Also an axial loading vector is present foot to head which has been shown to lead to hypertrophy of the cervical muscles and thoracic discs (Belavý *et al.*, 2013). As such investigations of an alternative platform, hyper-buoyancy flotation (HBF) was undertaken in this thesis.

Stature assessment

Hyper-buoyancy flotation offers a potential analogue for the investigation of spinal elongation countermeasures. It combines the principles of dry immersion, with the buoyant properties of hypersaline water that are used in restrictive environmental simulation therapy tanks (Jonsson and Kjellgren, 2014) that are synonymous with the effect experienced with the dead sea. By providing a buoyant platform that is separated from the water the participant is unloaded without an induced axial vector and accessible. This thesis includes the first trials using this analogue to investigate the effects of unloading on elongation. In the first experimental Chapter (Chapter 3) 4h and 8h static HBF assessments were performed using stadiometry to assess elongation during and following HBF. Both 4h (1.7±0.8cm) and 8h (2.2±0.6cm) trials resulted in significant elongation that was either equal to or greater than that reported from sleep studies (Tyrrell, Reilly and Troup, 1985) and head down tilt (Styf *et al.*, 1997). No comparable data exists on stature for dry immersion due to issues measuring the participant whilst immersed. Several studies have also investigated the effects of spinal traction upon stature, a forceful method of inducing spinal elongation. In a study investigating 0, 30 and 60% body weight traction, applied via a pneumatic split traction table for 42 minutes, an 0.61cm, 0.57cm and 0.71cm stature elongation was reported, acting as a surrogate measure for spinal length (Rodacki *et al.*, 2007). Another study using 30% bodyweight traction for 25 minutes reported an increase of 0.89cm after only 25 minutes. Differences could as one author suggests be due to when stature measurements were taken (Rodacki *et al.*, 2007), which could affect preload on the IVDs and viscoelastic response (Vergroesen *et al.*, 2016).

Stature measurement in this thesis was performed with a commercial stadiometer. However scientific investigations measuring stature have made use of a modified stadiometer which takes into account control of posture and curvature of the spine (Rodacki *et al.*, 2001). Standard deviation of measurement was similar between stadiometers, though it is marginally higher in the commercial stadiometer (0.48 vs 0.7mm). The mean detectable change of stadiometry in this thesis was (0.29mm), which is lower than the recorded differences post HBF, thereby facilitating investigation of load/unloading effects. However, the range between repeat measures is far lower with the modified stadiometer compared with the commercial stadiometer (0.5mm vs. 2mm). Therefore, a recommendation for future unloading studies is to utilise this modified stadiometer and introduce, where possible, the same rigour of measurement to spaceflight stature assessment.

For determining effect of unloading on the spine, stature was the principle quantity for effect, with a consistent increase of ~2.1cm on average in stature across studies, following 8h HBF. Whilst imaging modalities were employed in Chapters 4-7, only Chapter 6 provides imaging data on the effects of HBF unloading on the spine, where pre-and post HBF measurements of lumbar and cervical anterior IVD heights were assessed with ultrasound. Significant increases in the anterior IVD height were observed in three cervical and one lumbar disc following 8h overnight HBF, with non-significant increases in all other discs compared with pre. The sum of the measured cervical disc heights accounted for 10% of the stature elongation, with the measured lumbar heights accounting for a further 15% of the stature elongation. The cervical measurement contribution is in line with the 20% proposed in a study of diurnal height, from miscellaneous sources (which included cervical discs) to total stature elongation (Wing et al. 1992). However, the contribution from the lumbar measurements is less than the 40% that would be expected from the same study, suggesting the anterior measurements taken may not sufficiently account for elongation effects. Differences could be due to the imaging window employed, this thesis replicated the ISS protocol (Marshburn *et al.*, 2014b) that scans the spine anteriorly whereas others have scanned posteriorly lumbar height from the L1-L4 process's (Ledsome et al. 1996). An advantage of scanning posteriorly is signal clarity, as bowel gas can distort images of the lumbar IVD, the disadvantage is the architecture of the connecting lumbar structures obscures the IVD. This current study does however provide the first published data on the NASA protocol that recorded unloading of both cervical and lumbar discs. Ultrasound offers a portable modality that from the unloading observations in Chapter 6 is recommended to be repeated in space to provide further knowledge to the cervical and lumbar IVD swelling, as no actual evidence in-flight of IVD swelling has been recorded, just inferred. With the combined ultrasound measures and consistent stature elongations, it is reasonable to assume that HBF induces significant stature elongation, that is facilitated through IVD swelling.

In order to better establish an evidence base to support the use of HBF as a microgravity analogue platform, the length of HBF requires extending to that used in short term (3-7 days) HDT (Styf *et al.*, 1997) and dry immersion (Treffel *et al.*, 2016) studies, where the adaptations to unloading in the spine can be observed, not just the acute impact of load/unloading in this thesis. Furthermore, pre-and post-imaging assessments are required to identify if IVD swelling is occurring and using ultrasound whether there is a plateau after several days as stature measures alone are insufficient to determine both the effect and mechanisms behind unloading and have potential for measurement error and bias. Alternative modalities of stature assessment including aforementioned modified stadiometers (Rodacki *et al.*, 2001) and camera systems identified for in-flight assessment on the ISS (Sudhakar *et al.*, 2015) should also be investigated for integration into future analogue studies.

Subjective findings

Subjective ratings of movement discomfort and body control were low throughout HBF, whereas reports from dry immersion have described discomfort from water compressing the chest (Barr et al. 2015; Andrade et al. 2014). In Chapter 4, lower back and neck pain was reported with >5h HBF, which could be linked to the IVD swelling. IVD swelling is purported to induce stretching of the surrounding soft tissue (Sayson and Hargens, 2008), as observed with prolonged unloading and reports of back pain in space (Wing *et al.*, 1991), dry immersion (Watenpaugh, 2016) and head down tilt (Styf *et al.*, 2001). A study with 3-day dry immersion found significant disc swelling through an increase in disc volume, with 92% of participants reporting back pain using a 0-10 visual analogue scale similar to that employed in Chapter 3 (Treffel *et al.*, 2016). In space, adoption of a fetal tuck position is often assumed to relieve back pain, as flexion on the spine loads the disc (Sayson *et al.*, 2013). However, in the first two Chapters and in HDT and dry immersion studies, participants were instructed to remain as still as possible. Thus, whether this back discomfort was associated with IVD swelling or the restraint in utilising muscle contractions to achieve an “equilibrium state” on the disc is unclear and could be investigated further to optimise analogue models and study design. Of note is that during the loaded condition in Chapter 4 and after 8h overnight HBF, reports of back discomfort were lower than without loading/movement.

Therefore, the notion that prolonged unloading of the disc, coupled with reduced muscle activation through restricted movement acts as a triggering factor for back discomfort is suggested. Back pain is however a multifaceted condition, that can incorporate several inputs including nociceptive, somatosensory and neurological (Flor, 2002). If HBF is to be used for future short-term studies (3-7 days), incorporation of specific assessments evaluating underlying mechanisms resulting in back pain should also be investigated. This could be done through a combination of techniques including visual analogue scales, as used in the current study, questionnaires including the Oswestry disability index (Davidson and Keating, 2002), somatic assessments through palpation of vertebral segments employed in dry immersion studies (Treffel *et al.*, 2017) and kinematic assessment with investigation of intervertebral motion (Breen and Breen, 2017). This list is not exhaustive, but based on the pilot studies could be readily employed into a future exploratory HBF study to determine suitable assessment techniques that are compatible with this platform.

Section 8.02 Effects of SkinSuit loading upon spinal elongation

The loss of axial loading in space has been attributed to the significant stature elongation experienced in astronauts (Thornton, Hoffler and Rummel, 1977) associated with IVD swelling and flattening of the spinal curves (Sayson *et al.*, 2013a). The resulting loss of mechanical stresses on the IVD leads to extracellular matrix remodelling, reduced protein uptake and apoptosis pathway signalling (Brisby *et al.*, 2010; Jin *et al.*, 2013). Combined with the atrophy of paraspinal extensor muscles (Chang *et al.*, 2016; Belavý, Gast and Felsenberg, 2017) astronauts have an increased susceptibility post-flight to injury and disc herniation (Johnston *et al.*, 2010; Sayson *et al.*, 2013a). Thus, reintroduction of axial loading in space to impart mechanical load upon the IVDs is required for viable long term human spaceflight and colonisation efforts on partial gravity environments. The SkinSuit is a proposed spaceflight countermeasure to impart axial loading in space, that has been developed to its current version (Mk VI) to be tolerable for long-term wear and is deployable in microgravity. The pilot studies in this thesis provide the first data of the effect of the suit's axial loading, upon markers of spinal elongation.

Overview of evaluation

Chapter 4 and 5 explored the effects of 8h wear of the SkinSuit, either after daywear (Chapter 4) or overnight (Chapter 5) wear vs. a controlled unloaded condition, which in Chapter 4 was gym clothes and in 5 was the SkinSuit in an unloaded configuration. Chapters 6 and 7 investigated the effects of 4h SkinSuit reloading after 8h HBF unloading to improve the application to spaceflight operations, Chapter 6 was a feasibility study of the design with no control as performed in

Chapter 3, whilst Chapter 7 compared the effects of unloaded vs. 4h reloaded. Employment of imaging was done in all studies which evolved in line with study objectives and garment optimisation, DEXA in Chapter 4, MRI in Chapter 5, ultrasound in Chapter 6 and MRI and QF in Chapter 7. Stadiometry was utilised across all studies as a consistent, surrogate measure of spinal elongation.

Effect of SkinSuit loading upon stature

The average loading imparted by the SkinSuit increased throughout the studies with the manufacture of new suits from 0.13Gz - Chapter 4, 0.15Gz - Chapter 5, 0.17Gz - Chapter 6 and 0.19Gz - Chapter 7. With SkinSuit loading, a 4mm attenuation in stature was reported after 8h HBF in Chapter 4, whereas in Chapter 5 an 8mm reduction was noted after 8h overnight HBF. With reloading of the SkinSuit in Chapter 6, an immediate reduction of 1mm was recorded at the beginning of reloading with only minor increases after four further hours reloaded on the HBF. In Chapter 7 a 4.5mm reduction in stature was recorded with immediate donning of the suit, with no further measures taken post 4h reloading, owing to priority for direct transport to imaging. Whilst this means there was variation in the study protocols, the trends across the Chapters indicate SkinSuit loading is acting to attenuate/prevent elongation.

The amount of compression observed across the studies is similar to other research on the effects of additional loading (through backpacks) on stature. Though due to a number of protocol differences between studies these findings are not directly comparable. Acute backpack loading with 15% bodyweight induced a 5mm reduction in stature, after 20 minutes of loading (Chow *et al.*, 2011). However the time of day was not controlled in that study, whereas it was in this thesis, therefore a confounding effect of preload cannot be discounted, which could affect the compressibility of the discs when comparing studies (Schmidt et al. 2016). Also, how loading was applied was different, with SkinSuit loading applied whilst laying down for either 4h or 8h (apart from initial donning), whereas with the backpack study it was when upright for 20 minutes, thus it is 1G + 15% bodyweight. Chapter 7 recorded a stature attenuation of 4.5mm directly after donning the SkinSuit, following 8h overnight unloading which may more closely represent the acute compression observed in the backpack study, as opposed to the prolonged loading effects upon stature in Chapters 4-5.

The prolonged wear of the SkinSuit either attenuated stature compared to control conditions or prevented further elongation after 8h of HBF unloading. Diurnal stature investigations have demonstrated 54% of stature elongation resulting from 8h sleep is lost within the first hour of 1Gz weight bearing (Tyrrell, Reilly and Troup, 1985). Whilst the SkinSuit only loads with 0.13-0.19Gz, it does attenuate stature in a comparable manner, when the degree of loading is factored in. Deployed in space it is therefore hypothesised the SkinSuit will attenuate stature proportionally to the low-level loading imparted. Reports from Thomas Pesquet's mission will provide data on the utility of the SkinSuit to attenuate stature elongation.

Effect of loading on IVD height

The lumbar and thoracic regions contribute 80% to stature elongation, with cervical and miscellaneous sources contributing the remaining 20% (Wing *et al.*, 1992). In Chapter 4 use of DEXA indicated a significant compression of 1.7mm at the centre of the L1/L2 IVD space with SkinSuit loading. This corresponded to ~50% of the gross stature attenuation. The MDC of DEXA was 0.32mm so whilst the clarity of images was not optimal, it does appear to permit measurement of intervertebral space in settings where MRI or CT scanning is not permissible. In Chapter 5, a 3mm reduction in lumbar length was observed, with non-significant reductions in thoracic (8mm) and cervical length (2mm) with >8h SkinSuit loading using MRI. Average lumbar IVD height at leach level L1-S1 was also partially attenuated by 0.2-0.3mm. However, the MDC of 0.25mm coupled with the low sample size makes these results only suggestive of a loading effect.

In Chapter 6, ultrasound measured an increase in anterior disc height at L2-L3 (1mm) and L4/L5 (0.5mm) with a decrease at L5-S1 (0.8mm) with immediate SkinSuit donning. With prolonged reloading, no observable effect was observed following initial SkinSuit donning, this might be because changes imparted by the low-level axial loading were either too small to be detected (even though MDC was between 0.01-0.02mm) or inadequate sample size. The minor increases in lumbar anterior disc height at these levels were also observed with MRI (in Chapter 5), however as discussed, the MDC was higher than the observed changes so only a trend for a small compression on the IVD can be deduced. In Chapter 7 with 4h of SkinSuit reloading lumbar length was not different between conditions (only a 0.4mm reduction), whereas a trend for a minor 0.2mm reduction in average IVD height was recorded at L3/L4 and L4/L5, which are the main areas for disc herniation (Johnston *et al.*, 2010). However, MDC's of IVD height were between 0.1-0.32mm meaning again these small trends are suggestive of a minor reloading effect. A trend for an increase in the CSA of the lower lumbar levels (L4-S1) was also observed with a reduction in L2/L3 volume which was similar in magnitude to a study investigating the impact of wearing 17.5% bodyweight vest during walking (Lewis and Fowler, 2009). The magnitude of IVD compression with MRI studies 0.2mm-0.3mm is consistent between Chapter 5 and 7. It is less than that reported with compression harnesses of 0.8mm, though the harness loaded to 50% bodyweight (Kimura *et al.*, 2000, 2001) compared to less than 20% bodyweight of the Mk VI SkinSuit.

An interesting observation between Chapters is the significant attenuation in lumbar length in Chapter 5 but not 7, similarly with use of the compression harness, where disc height was reduced but lumbar length was not (Kimura *et al.*, 2000). This could be due to an interaction of magnitude and time of compression or differences in participants (Appendix). In Chapter 5 it was 8h's plus transport to the MR, whilst in Chapter 7 it was 4h and in the harness example it was 11 minutes. Whilst it would then be argued that a higher loading is required to induce significant IVD compression, the prolonged application of higher magnitude axial force is not recommended for countermeasure development at this stage. A study which used 50% bodyweight creep-loading in a supine position in-vivo to measure the diffusion of solutes into the disc also found after 4.5h that there was no detectable change in disc deformation but there was a reduction in the solute flow into the disc (Arun *et al.*, 2009). Therefore, it is suggested that rather than increase the magnitude of loading, that the effect of cumulative SkinSuit wear upon lumbar disc geometry, in both long duration analogues and spaceflight should be investigated, to determine if the reimporting of a low-level axial loading cycle acts to support the IVDs.

Effect of loading upon lordosis also varies between research groups (Kimura et al. 2000; Shymon et al. 2014; Lee et al. 2003) and in this thesis, with Chapter 5 observing an increase in 4 out of 6 participants with loading and in Chapter 7 no effect at all. A study in children wearing backpacks found 50% of participants had an increased lordosis with loading, with variability suggested to relate to compensatory changes in participants posture (Neuschwander et al. 2010). The question of whether the measurement of lordosis is important when investigating the effects of load/unloading requires further study. Based on its measurement in this thesis it is unclear, potentially due to the lack of pre-and post HBF imaging of lumbar lordosis, which would strengthen the case for its inclusion in future studies if undertaken. Also, due to the heterogeneity between studies controlling participants posture prior to scanning, either through a supported recumbent/seated or standing assessment would enable clearer comparability between studies and determination of an effect axial loading on lordosis. Consistency with transport to imaging was an issue between studies that may also have influenced results. In Chapter 5 time and distance to the MRI scanner following unloading on the HBF was an issue, as participants had to be transported across London due to the decommissioning of the local MR scanner. This issue was optimised in Chapter 4, 6 and 7 by having the HBF within direct access of imaging, through either supine transport or portable imaging equipment.

<u>Effect of loading on lumbar kinematics</u>

Assessment of lumbar kinematics in the NASA study (Chang *et al.*, 2014; Sayson *et al.*, 2015) measured the IV-ROM and found a reduction post-spaceflight in the range of motion, for which the authors mistakenly claim as a measure of stiffness, which cannot be inferred in-vivo. In Chapter 7, measurement of intervertebral restraint using a surrogate measurement, laxity, was taken in combination with an array of other measures from terrestrial QF investigations to improve upon the NASA protocol (Mellor et al. 2014; Du Rose & Breen 2016; Breen & Breen 2017; Breen et al. 2015). Time to scanning was also standardised by building the HBF within the imaging centre. In the NASA study, it took three days from landing to perform the scanning on the astronauts which could have confounded their results due to the extreme Gz stress from re-entry and with immediate rehabilitation (Payne, Williams and Trudel, 2007). Lastly the NASA study used only standing QF, whereas passive recumbent scanning was used in Chapter 7 to mitigate the influence of muscle activity on intervertebral motion. These enhanced controls in this thesis mean that whilst results cannot be directly compared, they do provide the first data on the effects of axial unloading and reloading upon lumbar kinematics contributing to knowledge in this area.

The hypothesis was that as the NASA study had observed that unloading induces a decreased range of motion of the intervertebral discs and reloading the spine would act to reduce measures of intervertebral restraint. Reloading prior to flexion in vivo or "preloading" axially had a small but non-significant effect of reducing measures of intervertebral restraint, which could be related to the consistent, small compression of lumbar IVDs of 0.2mm. During extension, a decrease in measures of intervertebral restraint and variability in how the motion was shared across the vertebral levels was suggested. This may be due to magnitude of load imparted by the SkinSuit in a recumbent position, as at lower loads ligament tension acts to increase the intradiscal pressure during flexion above that observed in a neutral posture, whilst at higher loads this difference is markedly reduced (Adams *et al.*, 1994). These results may also be a product of the combined compressive and bi-directional elastic properties of the SkinSuit and how this may result in a small confounding activation of extensor muscles at the extreme range of motion in a passive recumbent movement when muscle activity is minimal.

Application of compressive forces have been found to increase spinal extensor activity particularly at the upper thoracic region (Callaghan and McGill, 1995). During development of an exoskeleton to support movement and transition of compressive forces, it was observed that the application of a pull spring at the thorax and a push spring at the lumbar region worked to decrease extensor muscle (erector spinae) activity at the thorax (T11) by 40% and at the lumbar (L3) by 9% whilst at a 45^0 flexion (Zhang, 2014). This in turn reduced the intervertebral reaction torque mainly through application of the push spring at the lumbar level. Developments of this system stemmed from a biomechanical model made in OpenSim which incorporates the musculoskeletal insertions. However, it did not factor in the apophyseal joints, which also act to resist the compressive force across the disc (Adams *et al.*, 1994). This does ask the question if the SkinSuits elastic design is affecting the musculoskeletal activation during specific loading tasks, which incur greater risk post spaceflight due to deconditioning/swelling i.e. flexion based tasks (Adams, Dolan and Hutton, 1987; Belavy *et al.*, 2016) or complex movements incorporating rotation (Schmidt *et al.*, 2007). This activation, which could be tested through electromyography, may act to ‘protect’ the discs, increasing the restraint on the IVDs and distributing loading. However, it is noted that the differences in QF outputs between conditions are lower than the MDC for these measures (personal communication with AECC imaging team, 2017). Given the low sample sizes firm conclusions cannot be drawn, however the notion that SkinSuit reloading acts to impart loading on the spine, is supported by these results. It is recommended that investigations of how SkinSuit elastic loading effects not only paraspinal muscular activity but also muscle tension are investigated both in passive, and active (dynamic and static) postures. This would aid in the further exploration of whether reloading/unloading effects the ability of the spine to compensate against both shear and compressive loading forces (Callaghan and McGill, 1995).

Finally, increases in IAP are linked to increases in spinal stiffness (Hodges *et al.*, 2005), with IAP increased through activation of abdominal muscles to support unloading of the spine during compressive motion (Stokes, Gardner-Morse and Henry, 2010). A SkinSuit study evaluating IAP in a passive state did not however find the suit to increase IAP (*Seghal et al - unpublished*). It could be argued though that as no measures were taken during an active flexion/extension task (Stokes, Gardner-Morse and Henry, 2010), the effect of SkinSuit compression may have been dampened down. However, this requires quantification to substantiate the effect of SkinSuit loading upon flexor and extensor activation and the concurrent interaction of IAP and trunk muscle activity during movement tasks.

Concluding remarks on SkinSuit loading

Together the results from stature and imaging studies suggest that the low level axial loading imparted by the SkinSuit is acting on the spine to reduce stature with a proportional effect upon the lumbar spine. The principal affects are observed with initial donning that appears to act to prevent further elongation thereafter. Whilst Chapters 5 and 7 are not directly comparable, prolonged wear above 4h does not appear to result in significantly greater benefits than that reported in 4h. Thus 4h wear appears sufficient to impart minor compression upon the lumbar spine. Whether this is enough to mitigate the effects of spaceflight however is unknown. Further studies are recommended with repeat cyclic on-off wear over several days (3-5 days) to determine cumulative benefit, reflective of the operational application in space. Results from Thomas Pesquet's mission will enable greater clarification of the design of these future evaluations. Ultimately more data on the effects of prolonged spaceflight on the spine is needed, using an improved array of measures pre-and post-spaceflight including kinematics (that have been tested in this thesis), coupled with in-flight measurements of IVD height which could be done through the ultrasound protocol used in this thesis and in space. This data in conjunction with monitoring of the application of SkinSuit reloading in space with pre vs. post flight measures would provide the rationale for countermeasure utility.

Section 8.03 SkinSuit design and future considerations

There was considerable variation in the loading imparted by the SkinSuit between studies which came closer to the design level of 0.2Gz over the course of the thesis (0.13Gz-0.19Gz). This may in part be due to SkinSuit fit and how it anchored to the body, either through errors in measurement, fluctuation in weight or translation of measurements into the tailoring process. As part of a materials investigation to improve the loading and its consistency imparted by SkinSuit technology, suit measurements were determined using 3D body scanning and NatickMSR (National Soldier Research Development and Engineering Centre, Natick, Massachusetts, USA) software (Kendrick, 2016). Four SkinSuits prototypes were manufactured from 3D scan measurements which led to no tailoring or fitting issues. This was also done for the fitting of Thomas Pesquet for his SkinSuit.

These prototype suits were built on the GLCS design but composed of both an elastic skinsuit and loading exoskeleton, which was designed to impart far higher loadings than the current Mk VI version providing 0.67-0.84Gz, which makes direct comparisons difficult. However, with this higher loading, discomfort was far greater with two participants unable to complete a 4h unloading vs. loading observation to determine effect on stature. This was performed in a study design similar to Chapter 4 for 4h supine. Due to a 50% non-finishers rate (2 out of 4) owing to severe discomfort of wear, no comparable data is available to support the use of increased loading to mitigate stature elongation. However, the design improvements that went into the SkinSuit component of these prototypes can assist in explaining why the designed loading of the current Mk VI SkinSuit may have fluctuated and how to mitigate this. The Mk VI SkinSuit is designed to impart 0.2Gz as modelled by the full stretch of its elastic components, that have a high material tension vertically and are made of stretch resistance material that does not degrade observably over time, however average loading did not reach this 0.2Gz. This is ascribed to improper suit fit and reduced anchoring affecting the loading, whereby the multiple cumulative stages merge together where suit anchoring is lost (Waldie and Newman, 2011; Kendrick and Newman, 2014). Rather than several stages loading the body, it becomes a single stage garment with sub-optimal fit and adherence. Optimisation of the current SkinSuit is therefore under consideration using underlays of silicone stripes to adhere to the body, the position of these stripes could aid in the load distribution of the spine and potential muscular activation strategies. As such further design optimisation is recommended, supported through modelling studies on the optimal placement of anchoring (Zhang, 2014).

The importance of ensuring all constituent components of the SkinSuit and future designs are compatible with the imaging modality will be a key consideration. To support further in-orbit assessments it is first recommended that pre-and post-imaging is taken using properly standardised protocols to properly establish a baseline comparison as so far only ultrasound data is available in this paradigm. Secondly the refinement of design to allow access to the lumbar spine is recommended (i.e. by extending the front zip), thereby allowing direct access to the area without the need for disruptive doffing/donning. This would allow multiple ultrasound investigations over time whilst wearing the SkinSuit both in space which has not been done and on the ground. Thirdly, it is paramount that advanced imaging modalities such as MRI are able to be integrated. MRI was used twice in this thesis providing critical insight in the effects of load/unloading. In one subject, a flaw was detected in a newly manufactured Mk VI SkinSuit (Chapter 7), whereby a metallic contaminant had been sown into the zip. This disrupted the scan and whilst not impeding some measurements it did distort scanning (Figure 38).

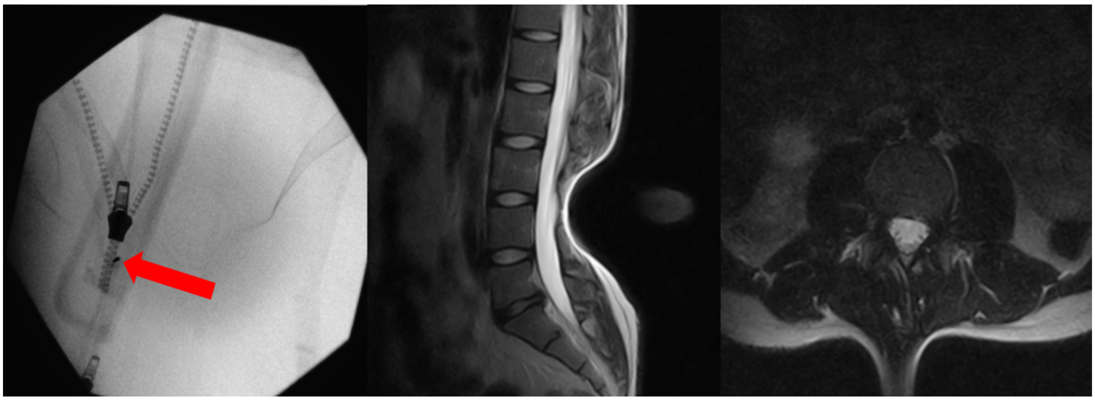


Figure 38. Fluoroscope image of a metallic contaminant that had been sown into a SkinSuit accidentally that caused distortion of MR images. Image credit AECC, Bournemouth, UK.

Section 8.04 Further recommendations for future assessments

Assessment of spinal length and stature

The use of manual measurements of height present issues both with stature measurements recorded during ground analogues (including this projects use of HBF), but also with those reported from space, where crew members would mark their colleagues respective head and foot position against the bulkhead position (Thorton and Moore, 1987). Whilst 3D laser body scanning would be an optimal modality for providing detailed anthropometric data (Kendrick, 2016), its current implementation in space presents logistical issues, as such a recent NASA study has assessed anthropometric (including stature changes) using a fixed digital camera system with markers on the ISS (Sudhakar *et al.*, 2015). Digital photography would provide a medium to assess the effectiveness of the SkinSuit in space but also in analogues.

Investigation of alternative methods to investigate regional changes in the spine in real time should also be pursued. Whilst ultrasound was successfully employed using an in-orbit protocol to measure anterior IVD height, further design refinements in the SkinSuit are needed to support integration with ultrasound. An alternative method is currently being investigated using conductive resistance displacement, where two sensors are attached to the region of interest i.e. L1-L4, separated by a conductive elastic material (piezoelectric) which is stretch sensitive. This can transmit results wirelessly through a low power wireless transmitter to record changes in resistance, thus displacement (Stoppa, 2016). This could be implemented into future SkinSuit design with other wireless technologies such as life-sensor monitoring to provide increased functionality of the SkinSuit, which will become increasingly more important in future exploratory class missions. This has been trialled with the HBF analogue, with further testing being planned.

Use of SkinSuit data to inform load/unloading models

Structural changes due to an increase in mechanical loading could be examined further than was possible in this thesis. A model on the effects of the additional loading provided by the SkinSuit would provide a platform for discussion on the magnitude of re-loading required at the lumbar level. Using the known loading of each SkinSuit, assumptions of loading at the lumbar level from IVD data could be used to inform computational models using comparative finite-element analysis, to investigate the effect of the SkinSuits loading upon lumbar spinal segments (Robson Brown *et al.*, 2014). Whilst a recent study in an animal model found cumulative loading accelerated lumbar disc degeneration (Bai *et al.*, 2017) this model consisted of loading with a collar whilst immobile, which would not be the situation of SkinSuit implementation.

Implementation of imaging

Further in-vivo imaging could also be performed including contrast MRI to investigate changes in the endplate of the discs, as mechanical loading is a major determinant of the endplates porosity and thickness (Zehra *et al.*, 2015). Damage to the endplate decompresses the adjacent disc accelerating delamination and degeneration (Stefanakis *et al.*, 2014). Additional loading over time/unloading could also affect the diffusion across the endplates into the nucleus pulposus of the discs (Rajasekaran, Naresh-Babu and Murugan, 2007), thus further imaging could help to quantify the effect of prolonged compressive SkinSuit loading on the endplate. Several analogue studies have also used MR spectroscopy to image changes in water content finding evidence after long term unloading of a reduction in the hydration of the disc (Sayson *et al.*, 2015; Treffel *et al.*, 2016). In future studies, it would also be advantageous to record the effect of cumulative acute bouts of SkinSuit wear (i.e. 4h's for 7 days) vs. a control on the effects of disc hydration to see if the provision of a cumulative, cyclic load has a beneficial effect for disc hydration.

The prolonged swelling of the IVDs in space could also potentially impart increased pressure on the endplates and vertebral bodies (LeBlanc *et al.*, 1994), this could lead to endplate damage and/or bone remodelling (Hansson and Roos, 1983) contributing to disc degeneration. As such whilst many spinal related spaceflight studies have focussed on the IVDs, further studies should also look to examine potential changes of extended unloading on the vertebral endplates and geometry. This would aim to determine if this is a potential risk factor for long term exploratory missions including ESA's planned lunar village and NASA's roadmap to Mars. In upcoming commercial spaceflight, several operators have sought to protect their participants by altering the magnitude of the G load from the z axis to the x axis. The implications on those with degenerative discs in a prolonged seated posture which affects the degree of stretch on the anterior/posterior annulus (Newell *et al.*, 2017) with increased G forces is unknown, which means there is the potential for an increase in mechanical failure of these sites. Therefore, as the participants are not screened or graded prior to flight for degeneration (unless it is in their medical history), further work investigating the effects of varying both the magnitude and axis of G on mechanical loading of the disc in both normal and degenerated discs is an area for future investigations.

<u>Potential terrestrial applications for further research</u>

An alternative treatment in the management of children with cerebral palsy has investigated the effect of suit therapy. Suit therapy is a spin off from the Russian' Pingvin suit and has been investigated as a potential rehabilitation tool through dynamic proprioceptive correction (Semenova, 1997). However a systematic review of four randomised control trials found considerable heterogeneity between trials with only limited efficacy for the utility of suit therapy (Martins *et al.*, 2016). One trial found a small increase in the mechanical efficacy in the group which used suit therapy (Adeli suit), however this was only in those with pre-existing good motor control in terms of optimisation, not increasing their gross motor skills (Bar-Haim *et al.*, 2006), with improvements seen in both the control (physiotherapy) and suit therapy groups, making it difficult to infer effectiveness of use. This does however provide a route-way for research to investigate in healthy controls who have neuromuscular deconditioning whether the addition of axial loading could act to improve both neuromuscular training/recovery and in particular trunk stability as this is an identified area of rehabilitation for astronauts returning after long duration spaceflight (Chang *et al.*, 2016) and after long term bed rest (Belavý, Gast and Felsenberg, 2017). An 8-week bedrest study rehabilitation program compared two methods of trunk rehabilitation, a trunk flexor strength program and a specific motor control program, both were successful at restoring the cross sectional area of the multifidus, with greater preservation of the psoas muscle in the flexor program (Hides *et al.*, 2011). The authors also noted a significantly higher reduction in disc volume and anterior disc height in that program, which led them to conclude the specific motor control program might be preferential to avoid excessive compression. Thus, a combined specific motor control program with the low axial loading of the SkinSuit might offer an ideal compromise between these training programs, to aid in trunk rehabilitation and distribution loading across the IVD.

Reflections on the Mk VI SkinSuits parallel implementation with the international space station

Following parabolic flight testing in 2014 to assess operational suitability of the SkinSuit (Figure 39), the MK VI SkinSuit was successfully incorporated into Andreas Mogensen's 10-day technological demonstration mission (IRISS) in September 2015. During this mission, he wore the SkinSuit on two occasions assessing don/doffing ability, the SkinSuits effect on the microbe environment and exercise compatibility. The SkinSuit then flew to the ISS for a second time in November 2016 for 6-months, where it underwent further evaluation with ESA Astronaut Thomas Pesquet during his mission (PROXIMA). Results from his mission are currently being evaluated by ESA. Data from these studies and recommendations will seek to aid in future discussions regarding SkinSuit technology.

Figure 39. Team SkinSuit after our first parabolic flight prior to Andreas and Thomas ISS missions. Thank you to everyone on the project! Image Credit: ESA, CNES & Novaspace.

Section 8.05 Conclusion

Based on the evidence presented in the literature, the IVDs are more prone to herniation following prolonged swelling. Long-term unloading associated with spaceflight induces significant elongation and a 4-fold increase in this IVD herniation risk, thus the need for further understanding of the role of load and unloading the disc for the development and evaluation of countermeasures is required. The body of work in the thesis has contributed to this knowledge, by investigating a potential novel analogue platform (HBF), the effects of unloading, loading and reloading with a proposed spaceflight countermeasure (the Mk VI SkinSuit) upon stature, spinal geometry and kinematics and provided new data, using a NASA ultrasound protocol on the effect of unloading on the cervical spine.

The Mk VI SkinSuit imparts a low-level axial load, close to its designed 0.2Gz, which can be worn for long periods (>8h) without significant hindrance, including during sleep. Data gathered from these pilot studies suggest SkinSuit loading to have an observable effect attenuating stature elongation. Imaging data shows a tendency for the SkinSuit to impart a minor loading effect, upon the lumbar intervertebral discs in both static (MRI) and dynamic assessments (quantitative fluoroscopy).

The short periods of HBF unloading and reloading with the SkinSuit in these pilot studies offer insight into the effects of spaceflight, but are not truly analogous of the long-term unloading encountered, nor the operational reality of a daily countermeasure program, where cumulative implementation may have a different impact of the spine. A longer duration flotation with countermeasure implementation and assessment, combined with imaging data from an astronaut pre, during and post spaceflight who has used the SkinSuit will offer further insight into both the unloading and reloading effects upon the spine and the utility of SkinSuit technology. Whilst sample sizes used in this thesis are similar to those employed in human spaceflight research analogues, the low numbers mean clear conclusions are difficult to draw at this time. Future studies should seek to improve the power of the studies with rigour in both the standardisation and implementation of measurements.

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Appendix

ISS pre-flight back pain questionnaire (example)

In-flight Back Pain Questionnaire

1. Do you have back pain now?

   Yes ☐ No ☑

2. Since you last completed this questionnaire (or since arriving at the ISS):

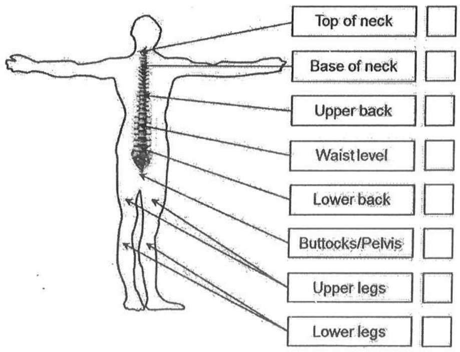
   - I have started to have back pain ☐
   - My back pain has decreased ☐
   - My back pain has increased ☐
   - I have stopped having back pain ☐
   - Nothing is different ☑

3. If you have or have had pain, please describe it.

   - It's mild, but has not been constant ☐
   - It's mild and has been constant ☐
   - It's moderate, but has not been constant ☐
   - It's moderate and has been constant ☐
   - It's severe, but has not been constant ☐
   - It's severe and has been constant ☐

4. At its worst, how much does it hurt (0 being very mild, 10 being extremely painful) 0

5. Using the image below, please indicate the location of your pain by ticking the boxes.



6. Have you taken any pain killers since you last completed this questionnaire (or since arriving at the ISS)? If yes, when and how many? No

7. Please use the box below to provide any additional comments.

Subjective rating scales and body pain map

Rating of Perceived Exertion (BORG) Scale

6	NO EXERTION AT ALL
7	EXTREMELY LIGHT
8	
9	VERY LIGHT
10	
11	LIGHT
12	
13	SOMEWHAT HARD
14	
15	HARD (HEAVY)
16	
17	VERY HARD
18	
19	EXTREMELY HARD
20	MAXIMAL EXERTION

Thermal Comfort ASHRAE Scale

+3	Hot
+2	Warm
+1	Slightly Warm
0	Neutral
-1	Slightly Cool
-2	Cool
-3	Cold

Movement Discomfort (Modified Corlett and Bishop Scale)

1) Nude comfort

2) Pyjamas, casual clothes

3) Formal attire

4) Minor discomfort if worn all day (16 h)

5) Too uncomfortable to wear all day

6) Too uncomfortable for 8 h

7) Too uncomfortable for 4 h

8) Too uncomfortable for 2 h

9) Too uncomfortable for 1 h

10) Too uncomfortable for 10 min

Body Control (Modified Cooper–Harper body control scale)

1) Unrestricted

2) Negligible constriction

3) Minimal compensation required

4) Minor but annoying constriction

5) Moderately objectionable constriction

6) Tolerable constriction

7) Difficult to control

8) Considerable compensation required

9) Intense compensation required

10) Body control lost

Pain rating scale

CHAPS BP Scale:

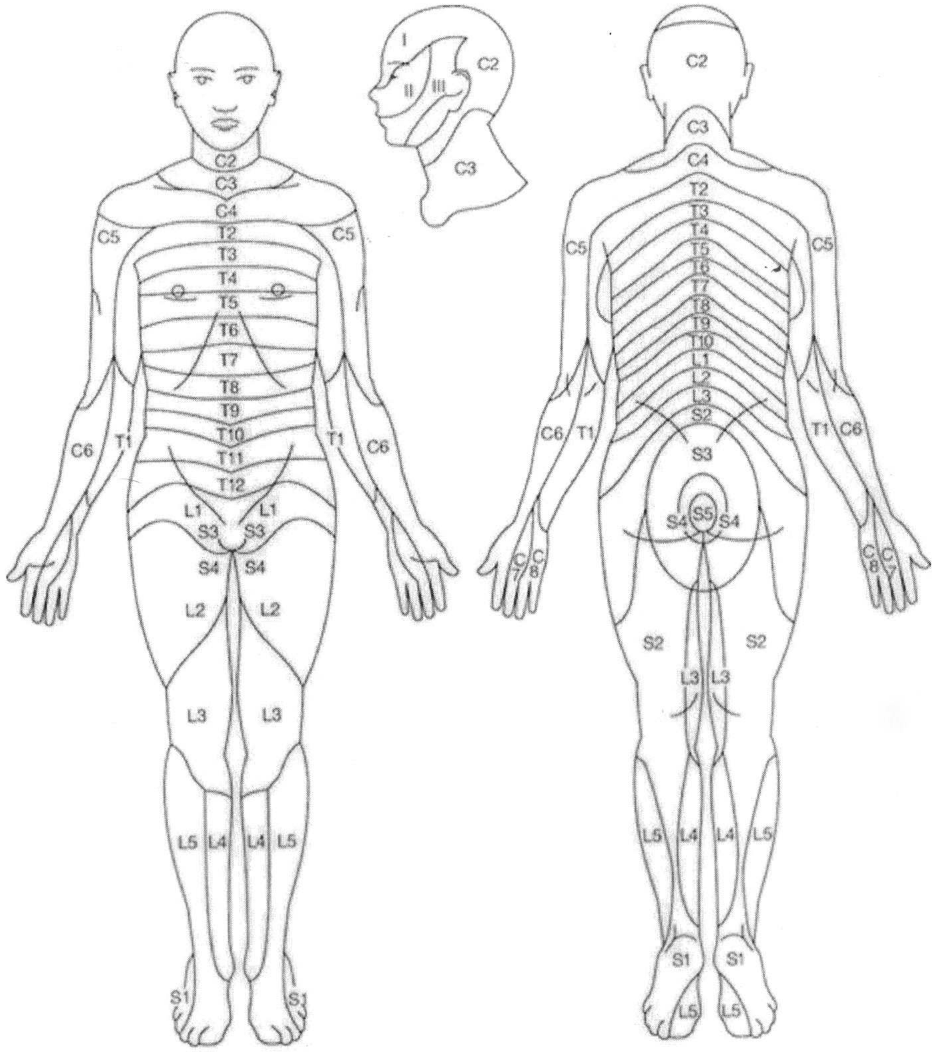
Body Pain Rating

Version: 20141007A

[0]	No observation
[1]	Sensitive
[1.5]	Stiffness
[2]	Mild Discomfort
[3]	Moderate Discomfort
[4]	Discomfort
[4.5]	Discomfort {not excruciating}
[5]	Pain / Severe Discomfort
[6]	Pain
[7]	Pain
[8]	Pain
[9]	Pain
[10]	Pain {non withstandable}

Pain map

Dermatome, Body map



Resource:
Grünenthal (2013) 'Dermatome map', Grünenthal Ltd. [Online], February.
Available from: http://www.change-pain.co.uk/useful-tools/dermatome-map/.

Participant utilisation

Table 13. List of participants common to each chapter

Thesis Chapter	Total Participants	Participants who partook in other studies (chapters)					
		Chapter	**3**	**4**	**5**	**6**	**7**
3	23	**3**		9	4	3	1
4	9	**4**	9		4	3	1
5	6	**5**	4	4		5	1
6	8	**6**	3	3	5		1
7	8	**7**	1	1	1	1	

Example of consent form

(All ethics applications are available online from the KCL-REMAS directory and/or for Chapter 7 from the NHS South West 3 Research Ethics committee)

CONSENT FORM FOR PARTICIPANTS IN RESEARCH STUDIES

Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

King's College Research Ethics Committee Ref: HR-15/16-2161

Title of Main Study: The effect and tolerability of the Gravity-Loading Countermeasure Skinsuit (GLCS) during prolonged wear and long duration floatation.

Thank you for considering taking part in this research. The person organising the research must explain the project to you before you agree to take part. If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.

I confirm that I understand that by ticking/initialling each box I am consenting to this element of the study. I understand that it will be assumed that unticked/initialled boxes mean that I DO NOT consent to that part of the study. I understand that by not giving consent for any one element I may be deemed ineligible for the study. — Please tick or initial ☐

Please tick or initial

- I confirm that I have read and understood the information sheet dated for the above study. I have had the opportunity to consider the information and asked questions which have been answered satisfactorily. ☐
- I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. Furthermore, I understand that I will be able to withdraw my data up 1st of February 2017. ☐
- I consent to the processing of my personal information for the purposes explained to me. I understand that such information will be handled in accordance with the terms of the UK Data Protection Act 1998. ☐
- I understand that my information may be subject to review by responsible individuals from the College for monitoring and audit purposes. ☐
- I understand that confidentiality and anonymity will be maintained and it will not be possible to identify me in any publications ☐

- I agree that the research team may access my general health questionnaire records for the purposes of this research project. ☐

- I agree that the research team may use my data for future research and understand that any such use of identifiable data would be reviewed and approved by a research ethics committee. (In such cases, as with this project, data would/would not be identifiable in any report). ☐

- I understand that I must not take part if I fall under the exclusion criteria as detailed in the information sheet and explained to me by the researcher. ☐

- I have informed the researcher of any other research in which I am currently involved or have been involved in during the past 12 months ☐

- I agree for the data collected in this study to be used to conjunction with current/future studies to characterise other data collected with myself as a subject pertaining to this skinsuit (GLCS) project (e.g. Imaging data of my spine) ☐

- I agree to the data being collected in this study to be used in the construction of external reports e.g. for the European Space Agency and understand and agree that data will be shared with collaborating partners on this project e.g. St Thomas Hospital & the Anglo European Chiropractic College ☐

- I consent to having myself recorded during this study in relation to the studies and consent to this material being used. ☐

- I give consent for the imaging scans taken in this study to be assessed by a trained radiographer/sonographer and used in reporting/analysis. As this is a research study not diagnostic, if it is advised by the radiographer/sonographer that an anomaly has been detected requiring a medical follow-up, I consent for the principal investigator to contact me in the first instance (in person or over the phone) and to provide my GP (details below) with further details/scanning information. ☐

____________	____________	____________
Your Telephone number	**GP Name**	**GP Surgery**
____________	____________	____________
Name of Participant	**Date**	**Signature**
____________	____________	____________
Name of Researcher	**Date**	**Signature**

Intra-observer data

Chapter 3 – Stadiometry

Breathing IN			
Measure 1	Measure 2	St Dev of me	Range
183.1	183	0.07071068	0.1
186	186.1	0.07071068	-0.1
159.9	159.8	0.07071068	0.1
171.8	171.7	0.07071068	0.1
168.1	168.3	0.14142136	-0.2
185.1	185	0.07071068	0.1
159.7	159.8	0.07071068	-0.1
171.6	171.9	0.21213203	**-0.3**
169.9	169.9	0	0
167.5	167.4	0.07071068	0.1
183.6	183.6	0	0
186.4	186.4	0	0
160.3	160.4	0.07071068	-0.1
172.1	172	0.07071068	0.1
168.8	168.6	0.14142136	0.2
184.9	185.1	0.14142136	-0.2
160.2	160.2	0	0
171.7	171.6	0.07071068	0.1
169.9	170	0.07071068	-0.1
167.7	167.7	0	0
172.415	172.425	**0.07071068**	0.3
ICC	0.999		
SEM	0.02mm		
MDC	0.06mm		

Breathing out			
Measure 1	Measure 2	St Dev of me	Range
182.9	182.9	0	0
185.7	185.7	0	0
159.7	159.7	0	0
171.7	171.6	0.07071068	0.1
168	168.1	0.07071068	-0.1
184.8	184.7	0.07071068	0.1
159	159.6	0.42426407	**-0.6**
171.4	171.7	0.21213203	-0.3
169.6	169.7	0.07071068	-0.1
167.3	167	0.21213203	0.3
183.3	183.4	0.07071068	-0.1
186.1	186.2	0.07071068	-0.1
160	160.1	0.07071068	-0.1
171.8	171.8	0	0
168.6	168.5	0.07071068	0.1
184.7	184.5	0.14142136	0.2
160	160	0	0
171.7	171.6	0.07071068	0.1
169.3	169.6	0.21213203	-0.3
167.4	167.2	0.14142136	0.2
172.15	172.18	**0.09899495**	0.6
ICC	0.999		
SEM	0.03mm		
MDC	0.09mm		

Chapter 4 – DEXA

Anterior height				Middle height				Posterior height			
Measure 1	Measure 2	St Dev of me	Range	Measure 1	Measure 2	St Dev of me	Range	Measure 1	Measure 2	St Dev of me	Range
10.1	10.2	0.07071068	-0.1	7.7	8.3	0.42426407	-0.6	6.7	6.6	0.07071068	0.1
9.7	9.7	0	0	7.4	7.4	0	0	6.3	6.3	0	0
11.5	11.7	0.14142136	-0.2	9.9	10.1	0.14142136	-0.2	6.3	6.3	0	0
7.1	10.5	2.40416306	-3.4	10.7	8.7	1.41421356	2	5.8	5.8	0	0
11.7	10.9	0.56568542	0.8	8.9	8.9	0	0	6.7	5.9	0.56568542	0.8
12.9	12.2	0.49497475	0.7	11	10.6	0.28284271	0.4	7.9	6.9	0.70710678	1
9.4	8.7	0.49497475	0.7	8.8	8.2	0.42426407	0.6	7.2	6.2	0.70710678	1
10.3	9	0.91923882	1.3	10.6	10.1	0.35355339	0.5	7	6.4	0.42426407	0.6
10.6	10.5	0.07071068	0.1	8.1	8.1	0	0	6.2	6	0.14142136	0.2
9.2	8	0.84852814	1.2	9.4	8.8	0.42426407	0.6	6.4	6.2	0.14142136	0.2
9.3	10.6	0.91923882	-1.3	9.1	8.7	0.28284271	0.4	6.1	6.4	0.21213203	-0.3
12.2	10.3	1.34350288	1.9	11.6	10.4	0.84852814	1.2	10.3	10.2	0.07071068	0.1
10.3333333	**10.1916667**	**0.68942911**	**3.4**	**9.43333333**	**9.025**	**0.38301617**	**1.2**	**6.90833333**	**6.6**	**0.25337993**	**1**
	ICC	0.906			ICC	0.912			ICC	0.794	
	SEM	0.21137496			SEM	0.11362096			SEM	0.11500211	
	MDC	0.58590151			MDC	0.31494123			MDC	0.3187696	

Chapter 5 – MRI

Anterior Height				Posterior Height			
Measure 1	Measure 2	Range	STD	Measure 1	Measure 2	Range	STD
7.44	8.12	-0.68	0.48083261	5.74	5.34	0.4	0.28284271
6.96	6.84	0.12	0.08485281	5.86	5.71	0.15	0.10606602
7.81	8.29	-0.48	0.33941125	3.36	2.94	0.42	0.29698485
7.81	7.77	0.04	0.02828427	3.95	4.44	-0.49	0.34648232
7.58	8.63	-1.05	0.74246212	6.06	6.04	0.02	0.01414214
7.72	7.05	0.67	0.47376154	5.71	6.25	-0.54	0.38183766
8.52	8.88	-0.36	0.25455844	4.79	4.71	0.08	0.05656854
7.49	6.83	0.66	0.46669048	4.27	5.14	-0.87	0.6151829
10.3	10.4	-0.1	0.07071068	8.03	7.54	0.49	0.34648232
9.55	10.3	-0.75	0.53033009	7.47	7.48	-0.01	0.00707107
10.6	10.5	0.1	0.07071068	6.07	5.98	0.09	0.06363961
9.4	8.8	0.6	0.42426407	6.11	6.67	-0.56	0.3959798
11.6	12.7	-1.1	0.77781746	8.25	8.73	-0.48	0.33941125
11	11.8	-0.8	0.56568542	6.95	8.06	-1.11	0.78488853
13.2	13.6	-0.4	0.28284271	8.36	9.58	-1.22	0.86267027
12.5	11.6	0.9	0.6363961	9.29	10.3	-1.01	0.71417785
14.8	15.4	-0.6	0.42426407	6.77	7.9	-1.13	0.79903066
13.4	14.2	-0.8	0.56568542	5.82	6.52	-0.7	0.49497475
14.2	13.7	0.5	0.35355339	5.31	6	-0.69	0.48790368
12.1	13.4	-1.3	0.91923882	6.76	7.19	-0.43	0.30405592
10.199	10.4405	1.3	0.42461762	6.2465	6.626	1.22	0.38501964

ICC	0.968	ICC	0.945
SEM	0.07595791	SEM	0.09029511
MDC	0.21054458	MDC	0.25028528

Spinal Length					Cobb angle			
	Measure 1	Measure 2	Range	STD	Measure 1	Measure 2	Range	STD
Cervical	122.4	121.3	1.1	0.8	11.43	8.65	2.78	1.96575685
Cervical	122.1	122	0.1	0.1	10.86	9.91	0.95	0.67175144
Cervical	123.9	124.4	-0.5	0.4	23.66	27.53	-3.87	2.73650324
Cervical	122	122.6	-0.6	0.4	21.91	14.54	7.37	5.21137698
Thoracic	303.7	303.2	0.5	0.4	4.14	3.61	0.53	0.37476659
Thoracic	300.5	299	1.5	1.1	3.53	3.68	-0.15	0.10606602
Thoracic	323.3	323.1	0.2	0.1	3.76	4.28	-0.52	0.36769553
Thoracic	325.7	326.1	-0.4	0.3	4.96	3.92	1.04	0.73539105
Lumbar	180.1	178	2.1	1.5	46.94	43.07	3.87	2.73650324
Lumbar	174.3	173	1.3	0.9	47.14	47.22	-0.08	0.05656854
Lumbar	181.7	180.3	1.4	1	34.72	38.49	-3.77	2.66579257
Lumbar	178.5	178	0.5	0.4	35.27	35.04	0.23	0.16263456
Average	204.9	204.3	2.1	0.4	41.0175	40.955	3.87	1.40537473

ICC	0.998	ICC	0.984
SEM	0.01788854	SEM	0.1777674
MDC	0.04958451	MDC	0.49274611

Chapter 6 –US

Cervical				Lumbar			
Measure 1	Measure 2	STD	Range	Measure 1	Measure 2	STD	Range
0.325	0.327	0.00141421	0.00	1.05	1.04	0.00707107	0.01
0.435	0.445	0.00707107	-0.01	1.26	1.21	0.03535534	0.05
0.423	0.423	0	0.00	1.17	1.2	0.0212132	-0.03
0.481	0.471	0.00707107	0.01	1.09	1.11	0.01414214	-0.02
0.457	0.424	0.02333452	0.03	1	0.99	0.00707107	0.01
0.51	0.495	0.0106066	0.02	1.22	1.26	0.02828427	-0.04
0.543	0.552	0.00636396	-0.01	1.06	1.1	0.02828427	-0.04
0.462	0.46	0.00141421	0.00	1.15	1.16	0.00707107	-0.01
0.451	0.431	0.01414214	0.02	1.07	1.05	0.01414214	0.02
0.485	0.49	0.00353553	-0.01	1.01	1.03	0.01414214	-0.02
0.545	0.58	0.02474874	-0.03	1.498	1.487	0.00777817	0.01
0.463	0.434	0.0205061	0.03	1.238	1.223	0.0106066	0.01
0.33	0.316	0.00989949	0.01	1.128	1.079	0.03464823	0.05
0.444	0.454	0.00707107	-0.01	1.02	0.974	0.03252691	0.05
0.418	0.426	0.00565685	-0.01	1.493	1.468	0.01767767	0.03
0.482	0.483	0.00070711	0.00	1.221	1.205	0.01131371	0.02
0.453375	0.4506875	**0.00791504**	**0.03**	1.167375	1.161625	**0.01038428**	**0.05**
	ICC	0.997			ICC	0.997	
	SEM	.004mm			SEM	.006mm	
	MDC	0.01mm			MDC	0.02mm	

Chapter 7 –MRI

Anterior Height				Middle Height				Posterior Height			
Measure 1	Measure 2	Range	STD	Measure 1	Measure 2	Range	STD	Measure 1	Measure 2	Range	STD
7.51	9.06	-1.55	1.09601551	10.9	9.78	1.12	0.79195959	5.47	5.15	0.32	0.22627417
8.29	8.14	0.15	0.10606602	9.86	9.99	-0.13	0.09192388	5.04	4.85	0.19	0.13435029
7.97	7.97	0	0	7.75	8.1	-0.35	0.24748737	3.53	3.49	0.04	0.02828427
8.71	8.98	-0.27	0.19091883	8.32	8.54	-0.22	0.15556349	3.97	3.74	0.23	0.16263456
8.7	10.4	-1.7	1.20208153	10.09	11.6	-1.51	1.06773124	6.52	5.73	0.79	0.55861436
9.2	8.5	0.7	0.49497475	10.7	10.7	0	0	5.35	5.35	0	0
7.99	8.4	-0.41	0.28991378	10.9	11	-0.1	0.07071068	4.62	4.49	0.13	0.09192388
8.59	8.33	0.26	0.18384776	10.5	10.4	0.1	0.07071068	5.07	5.28	-0.21	0.14849242
12.01	12.5	-0.49	0.34648232	13.2	13.7	-0.5	0.35355339	7.49	7.14	0.35	0.24748737
11.2	11	0.2	0.14142136	13.8	14.4	-0.6	0.42426407	5.54	5.54	0	0
9.37	9.75	-0.38	0.26870058	12.5	12.4	0.1	0.07071068	5.81	5.84	-0.03	0.0212132
9.82	9.8	0.02	0.01414214	12.6	12.7	-0.1	0.07071068	5.31	5.46	-0.15	0.10606602
12.2	15.9	-3.7	2.61629509	15.1	14.7	0.4	0.28284271	7.78	9.54	-1.76	1.24450793
14	12.3	1.7	1.20208153	14	14.5	-0.5	0.35355339	7.73	7.79	-0.06	0.04242641
16.1	16.3	-0.2	0.14142136	10.5	10.1	0.4	0.28284271	8.47	8.97	-0.5	0.35355339
15.3	15.7	-0.4	0.28284271	10.2	10.1	0.1	0.07071068	6.61	6.92	-0.31	0.2192031
17.7	19.1	-1.4	0.98994949	12.7	11.9	0.8	0.56568542	5.65	6.29	-0.64	0.45254834
17.4	17.1	0.3	0.21213203	12.1	12.5	-0.4	0.28284271	5	4.33	0.67	0.47376154
16.6	16.4	0.2	0.14142136	11.3	10.9	0.4	0.28284271	5.2	5.19	0.01	0.00707107
13.8	14.7	-0.9	0.6363961	9.95	10.2	-0.25	0.1767767	5.18	5.18	0	0
11.623	12.0165	1.7	0.52785521	11.3485	11.4105	1.12	0.28567114	5.767	5.8135	0.79	0.22592062
	ICC	0.952			ICC	0.983			ICC	0.933	
	SEM	0.11564728			SEM	0.03724696			SEM	0.0584781	
	MDC	0.32055791			MDC	0.1032433			MDC	0.16209303	

	cobb angle				Spinal length			
	Measure 1	Measure 2	Range	STD	Measure 1	Measure 2	Range	STD
	3.26	3.29	-0.03	0.0212132	121.3	122.2	-0.9	0.6363961
	2.33	2.35	-0.02	0.01414214	122.9	122.8	0.1	0.07071068
	18.77	18.99	-0.22	0.15556349	124.1	122.41	1.69	1.19501046
	26.72	26.62	0.1	0.07071068	125.8	125.3	0.5	0.35355339
	4.8	6.86	-2.06	1.45663997	302.2	303.3	-1.1	0.77781746
	3.8	3.91	-0.11	0.07778175	302.1	298.9	3.2	2.2627417
	2.63	2.62	0.01	0.00707107	322.9	323.5	-0.6	0.42426407
	3.7	3.73	-0.03	0.0212132	324.8	324.1	0.7	0.49497475
Lumbar	41.1	40.51	0.59	0.417193	180.4	178.2	2.2	1.55563492
Lumbar	46.91	47.06	-0.15	0.10606602	175.1	176.2	-1.1	0.77781746
Lumbar	35.52	35.82	-0.3	0.21213203	180.4	180.2	0.2	0.14142136
Lumbar	38.3	37.93	0.37	0.26162951	176.3	176.6	-0.3	0.21213203
	40.4575	40.33	0.59	0.24925514	178.05	177.8	2.2	0.67175144
		ICC	0.968			ICC	0.998	
		SEM	0.05			SEM	0.03004164	
		MDC	0.12			MDC	0.08327117	

Skinsuit Related Publications

2017

Attias, J, Carvil, P, Russomano, T, Evetts, SN, Waldie, J & Green, D (2017). The Gravity-Loading Countermeasure Skinsuit (GLCS) and its effect upon aerobic exercise performance. *Acta Astronautica*.

Carvil, PA, Attias, J, Evetts, S, Waldie, J & Green, DA (2017). The effect of the gravity loading countermeasure SkinSuit upon movement and strength. Journal of strength and conditioning research.

Carvil, PA, Jones, M, Home, D, Ayer, R, Osbourne, N, Breen A, Breen A, Russomano, T & Green, DA (2017). The effect of 4-hour partial axial reloading via the Mk VI SkinSuit upon recumbent lumbar geometry and kinematics after 8-hour hyper-buoyancy flotation. 2nd Human Physiology Workshop, Cologne, 2017 (Submitted)

Carvil, PA, Gkouvinas, T, Russomano, T, Breen A, Scott, J, Halson-Brown, H, Villa, A, & Green, DA (2017). The effects of the Mk VI SkinSuit on the spinal column after 8h Hyper-Buoyancy Flotation. Joint ISPG-ELGRA Workshop, Nice, 2017

Carvil, PA, Gkouvinas, T, Russomano, T, Breen A, Scott, J, Halson-Brown, H, Villa, A, & Green, DA (2017). The effects of the Mk VI SkinSuit on the spinal column after 8h Hyper-Buoyancy Flotation. Biology of Ageing Conference, Singapore, 2017.

Carvil, PA, Russomano, T, Halson-Brown, H, & Green, DA (2017). The effect of 4-hour SkinSuit induced partial axial reloading upon stature elongation and anterior intervertebral disc height as assessed by ultrasound after 8-hour hyper-buoyancy flotation. British Medical Society Ultrasound Workshop, Chelmsford, 2017

Seghal, A, Carvil, PA, Macbean V, Rafferty G, & Green, DA (2017). Effects of the Mk VI SkinSuit on Intra-Abdominal Pressure. Joint ISPG-ELGRA Workshop, Nice, 2017

Attias, J, Mileva, K, Russomano, T & Green, DA (2017). Axial body loading induces changes in the neuro-mechanics of human running. Joint ISPG-ELGRA Workshop, Nice, 2017

2016

Attias, J, Scott, J, Mileva, K, Russomano, T & Green, DA (2016). 'The effect of axial body loading on lower limb neuromuscular activity during static and dynamic exercise'. in Joint Life Science Meeting ‘Life in Space for Life on Earth’. 14th European Life Sciences Symposium 37th Annual International Gravitational Physiology

Carvil, PA, Rios-Kristjansson, JG, Russomano, T, Scott, J, Moore, AE, Dulnoan, D & Green, (2016). 'The effect of axial unloading and partial reloading via donning the SkinSuit on the thoracolumbar spine'. in Joint Life Science Meeting ‘Life in Space for Life on Earth’. 14th European Life Sciences Symposium 37th Annual International Gravitational Physiology Meeting, Toulouse, France, 5-10 June. **Young Researcher Award**

Carvil, PA., Russomano, T., Baptista, R., Jain, V., Lindsay, K., Subasinghe, T., Waldie, J & Green, DA (2016). Biomechanics and the cardiorespiratory responses to self-selected running speed in simulated altered gravities-a case study. The Biomedical Basis of Elite Performance (Nottingham, UK). Proc Physiol Soc . 35, PC34.

Rosado, H., Doyle, R., Franco, R., Negus, D., Davies, C.H.M., Green, D.A., Scott, J., Mogensen, A., Stabler R.A & Taylor, P.W. (2016). Metagenomic evaluation of the microbiological burden of a Gravity-Loading Countermeasures SkinSuit throughout an ISS mission. International Astronautical Conference, Guadalajara, Mexico, 26th-30th September.

2015

Attias, J, Carvil, P, Evetts, SN, Waldie, J & Green, D (2015). Effect of the gravity loading countermeasure SkinSuit (GLCS) upon aerobic exercise performance. *European Low Gravity Research Association 22nd Symposium, Corfu, Greece.*

Attias, J, Scott, J, Russomano, T & Green, DA (2015). The effect of the Mk VI gravity-loading countermeasure SkinSuit (GLCS) upon maximal aerobic exercise (VO_2max). *International Congress of Aviation and Space Medicine (ICASM), Oxford, UK*

Carvil P, Kristjánsson J, Attias J, Russomano T & Green D (2015). Elongation induced by four hours of hyper-buoyancy floatation (HBF). *European Low Gravity Research Association 22nd Symposium, Corfu, Greece.*

Carvil, P., Russomano, T., Baptisma, R., Jain, V., Lindsay, K., Subasinghe, T., Waldie, J and Green, DA. (2015). Biomechanics and the cardiorespiratory responses to self-selected running speed in simulated altered gravities – a case study. Royal Society Open Science *(Accepted with minor revisions).*

Green, DA., Kristjánsson, JGK., Frechette, A., Scott, JPR. (2015). The gravity-loading countermeasure SkinSuit attenuates stature elongation and back pain during 8 h of human spinal unloading. Programme of the 36th Annual International Gravitational Physiology Meeting. Ljubljana, Slovenia. June 7-12:1730.

Green, DA, Attias, J. Carvil, P, Evetts, S, Scott JPR. (2015). The Gravity Loading Countermeasures Skin Suit - a collaborative journey to the ISS. UK Space conference 2015.Liverpool, UK. July 13-15.

2014

Attias, J, Evetts, SN, Waldie, J & Green, D (2014). The effect of the gravity-loading countermeasure SkinSuit (GLCS) upon maximal aerobic capacity (VO_2max). *Aviation Space and Environmental Medicine* **85: 352.**

Carvil, P, Cinelli, I, Waldie, J & Green, D. (2014). Effect of Gravity Loading Countermeasures SkinSuit upon the haemodynamic responses to orthostatic stress. *Aviation, Space, and Environmental Medicine.* **89:** 272.

Green, D., Attias, J., Carvil, P., Fréchette, A., & Waldie, J. (2014). Gravity-loading countermeasure SkinSuit (GLCS) tolerability & performance in models of weightlessness. *In Charite in Space: 6th International congress of Medicine in Space and extreme Environments (ICMS).*

2013

Attias, J, Carvil, P, Evetts, SN, Waldie, J & Green, D (2013). The effect of the Gravity-Loading Countermeasure SkinSuit (GLCS) upon aerobic exercise performance. *Aviation, Space, and Environmental Medicine*. **84:**397-398.

Carvil, P., Russomano, T., Baptisma, R., Jain, V., Lindsay, K., Subasinghe, T., Waldie, J and Green, D. (2013). Respiratory response to Moon Running with a Gravity Loading Countermeasure SkinSuit. *International Congress of Aviation and Space Medicine (ICASM), Jerusalem, Israel.*

Carvil, P, Attias, J, Evetts, S, Waldie, J & Green, D. (2013). The Validity, Viability & Tolerability of a Gravity Loading Countermeasure SkinSuit (GLCS) during Ambulation & Resistance Exercise. *Aviation Space and Environmental Medicine.* **84:** 398.